INFORMATION TO USERS

This manuscript has been reproduced from the microfilm master. UMI films the text directly from the original or copy submitted. Thus, some thesis and dissertation copies are in typewriter face, while others may be from any type of computer printer.

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleedthrough, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send UMI a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, charts) are reproduced by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps.

Photographs included in the original manuscript have been reproduced xerographically in this copy. Higher quality 6" x 9" black and white photographic prints are available for any photographs or illustrations appearing in this copy for an additional charge. Contact UMI directly to order.

Bell & Howell Information and Learning
300 North Zeeb Road, Ann Arbor, MI 48106-1346 USA
800-521-0600

REPRODUCTIVE ECOLOGY OF THE SEA URCHIN *STRONGYLOCENTROTUS DROEBACHIENSIS*

by

Susanne K. Meidel

Submitted in partial fulfillment of the requirements

for the degree of Doctor of Philosophy

at

Dalhousie University

Halifax, Nova Scotia

November, 1998

DALHOUSIE UNIVERSITY

FACULTY OF GRADUATE STUDIES

The undersigned hereby certify that they have read and recommend to the Faculty of Graduate Studies for acceptance a thesis entitled "Reproductive Ecology of the Sea Urchin *Strongylocentrotus Droebachiensis*"

by Susanne K. Meidel

in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

Dated: November 20, 1998

External Examiner

Research Supervisor

Examining Committee

[redacted]

DALHOUSIE UNIVERSITY

DATE: 20 November 1998

AUTHOR: Susanne K. Meidel

TITLE: Reproductive ecology of the sea urchin *Strongylocentrotus droebachiensis*

DEPARTMENT OR SCHOOL: Biology

DEGREE: Ph.D. CONVOCATION: May YEAR: 1999

Permission is herewith granted to Dalhousie University to circulate and to have copied for non-commercial purposes, at its discretion, the above title upon the request of individuals or institutions.

Signature of Author

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Along the Atlantic coast of Nova Scotia, population outbreaks of herbivorous sea urchins *Strongylocentrotus droebachiensis* have caused major transitions in the shallow subtidal ecosystem from kelp beds to barrens (areas devoid of fleshy macroalgae). This thesis examines the effects of diet on the reproductive ecology of *S. droebachiensis*, and the influence of reproduction on the population dynamics of this species and hence the dynamics of the ecosystem. Using gonad index and histological methods, I demonstrated that *S. droebachiensis* has an annual reproductive cycle with spawning in March/April. A field study at two sites showed that sea urchins in kelp beds and grazing fronts (high-density aggregations at the borders of kelp beds) consumed a higher quality diet and had a higher gonad index than those in barrens. Dietary differences did not influence the quality of gonads. Analysis of size-at-age data indicated that diet had a small effect on adult growth rates, which were marginally higher in kelp beds and grazing fronts than barrens at one site. Juvenile growth rates showed the same pattern among habitats but differences were more pronounced. I measured the effects of food ration and feeding regime on reproductive maturation and growth of juveniles in a 22-month laboratory experiment. Sea urchins fed a high ration of kelp, with or without a protein (mussel flesh) supplement, and most of those fed a low ration of only kelp, were reproductively mature after 10 months. In contrast, sea urchins fed only coralline algae remained immature at the end of the experiment, and had lower survival rates than those in kelp-fed treatments. Growth rate and gonad index were high in sea urchins fed kelp and mussels, intermediate in those fed a high ration of kelp, and low in those fed a low ration of kelp. These results suggest that juveniles in kelp beds, because of a better diet, have a greater reproductive value than those in barrens. I used larvae produced by adults from this experiment to investigate the effects of parental nutritional condition and larval food (phytoplankton) ration on larval traits in a follow-on experiment. Larval food ration had a strong positive effect on the rates of development and metamorphosis; in contrast, parental nutrition had little effect on these variables. However, my results suggest that when planktonic food is abundant, larvae of well-nourished adults in kelp beds may metamorphose sooner than those of poorly nourished adults in barrens. Using models of fertilization kinetics and egg production based on data from this study and the literature, I predicted that sea urchins in barrens make the largest contribution to the overall zygote pool during the transition from kelp beds to barrens. Model results also suggest that temporal variation in zygote production alone cannot explain sea urchin outbreaks off Nova Scotia.

LIST OF SYMBOLS USED IN EQUATIONS

Symbol		Used in equation(s)
a	Proportion of adults	6.3, 6.17
A	Total area occupied by a subpopulation	6.19
b	Proportion of spawning adults	6.3
B	Gonad index	2.1
B_0	Gonad index in immature sea urchins	2.1
$c^{(n)}$	Proportion of sea urchins downstream of nth male	6.3, 6.4
C	Proportion of I that is spawned	6.6
d_0	Zero plane displacement	6.9
D	Test diameter	2.1, 2.2, 3.1, 4.1
D_0	Test diameter at settlement	3.1
E	Proportional sensitivity (elasticity)	6.21
f	Proportion of females	6.3, 6.17
F	Dry weight of eggs released	6.17
g	Constant in a logistic function	2.1, 3.1
G	Dry weight of sperm released	6.5, 6.6
h	Number of retrieved larvae and settlers	5.1
I	Proportion of total body wet weight that is gonads	6.6, 6.8
j	Body size (mid-point of 5 mm size class)	6.1, 6.7, 6.8
j'	Mean adult size (weighted)	6.2
K_z	Vertical eddy diffusivity	6.10, 6.11
l	Time taken by sperm plume to advect to point x_i	6.10
m	Number of males contributing sperm to plume	6.16

n	nth nearest male spawning upstream of the female	6.16
N	Asymptotic test diameter	3.1
$O^{(U)}$	Number of eggs spawned per unit area	6.17, 6.18
$p(j)$	Proportion of sea urchins in a size class of adults	6.2, 6.5, 6.17
q	Number of retrieved settlers	5.1
Q	Sperm release rate	6.1, 6.5
$r^{(n)}$	Distance to nth nearest downstream (upstream) spawning female (male)	6.3
R	Asymptotic gonad index	2.1
s	Height above the substratum	6.9, 6.11
s_0	Roughness height	6.9
S	Sperm concentration	6.1, 6.12, 6.16
t	Sperm-egg contact time	6.16
$\bar{u}$	Mean current velocity	6.1, 6.9
u_*	Friction velocity	6.1, 6.9, 6.10, 6.11, 6.14
v	Original model parameter in sensitivity analysis	6.21
v^*	Altered model parameter in sensitivity analysis	6.21
V	Total body volume	2.2
W	Total wet body weight	4.1, 6.6, 6.7
x	Point downstream of spawning male	6.1, 6.4
X	Original model output in sensitivity analysis	6.21
X^*	Altered model output in sensitivity analysis	6.21
Y	Age in years	3.1
$Z^{(U)}$	Number of zygotes produced per unit area by a subpopulation	6.18

$Z^{(L)}$	Number of zygotes produced on a coastal scale by a subpopulation	6.19, 6.20
$Z^{(T)}$	Total number of zygotes produced on a coastal scale	6.20
α_y	Diffusion coefficient along horizontal axis perpendicular to x	6.1, 6.4
α_z	Diffusion coefficient along vertical axis perpendicular to x	6.1, 6.10
β	Rate constant of fertilization	6.12, 6.13, 6.14, 6.16
δ	Spawning time of a male sea urchin	6.5, 6.8
η'	Transformed rate of metamorphosis	5.1
$\varphi(x)^{(P)}$	Proportion of eggs fertilized by a single male of size j' at point x_i	6.12
$\varphi(x)^{(M)}$	Proportion of eggs fertilized by multiple males of size j' at point x_i	6.15, 6.16
$\varphi^{(C)}$	Cumulative proportion of eggs fertilized by multiple males of size j'	6.15, 6.18
κ	von Karman's constant	6.9
ρ	Population density	6.3, 6.17
σ	Fertilizable area of an egg	6.13, 6.14
τ	Sperm half-life	6.12
υ	Sperm swimming speed	6.13

ACKNOWLEDGEMENTS

I would like to thank my supervisor, Dr. Bob Scheibling, for his guidance and support. Bob's great enthusiasm for my work was a constant source of motivation. I am grateful to Bruce Hatcher for useful discussions and comments on manuscripts. My other committee members, Drs. Tony Chapman, and Sandy Walde, also provided valuable suggestions and constructive criticism.

My study involved many hours in the field and the laboratory and I would like to thank the following people for their assistance: Toby Balch, Ian Dempsey, Michael Gedamke, Bruce Hatcher, Allan Hennigar, Danny Jackson, Mark Robinson, Alyson Shaw, and Lawrence Taylor. Fred Watts and Boris Worm collected large amounts of seaweed and mussels, and Donna Krailo provided phytoplankton cultures. Fred Watts also was a supportive dive officer and instructor. Special thanks go to Aurora Nedelcu who was always a reliable 'baby sitter'. Myriam Barbeau, Wade Blanchard, Heather Hunt, and Phil Yund provided advice on statistical analyses. Alma Cameron introduced me to the intricacies of histological techniques, which I fully explored in the laboratory of Dr. Brian Hall. I thank Art MacIntyre and Shawn Robinson who taught me how to age sea urchins. Myriam Barbeau, Bruce Hatcher, Anna Metaxas, and Phil Yund critically reviewed earlier drafts of my modelling paper, and Toby Balch, Jon Grant, Phil MacCauley, and Keith Thompson clarified aspects of hydrodynamics for me. Anna Metaxas and Phil Yund also commented on other manuscripts.

One aspect of grad school I thoroughly enjoyed was being a teaching assistant. I am much indebted to Chris Corkett and Angelica Silva who introduced me to this challenging and fun aspect of academia.

Special thanks go to Toby Balch and Heather Hunt, who were my lab mates throughout my time at Dalhousie. They provided a friendly atmosphere in the lab and

were excellent lab mates all around. A big thank you also goes to Aurora Nedelcu who was there for me as a friend and always happy to celebrate any number of small academic successes. And thank you to Thomas and John - I wouldn't be here without you.

Ein riesiges Dankeschön geht auch an meine Eltern und meine Schwester mit Familie, die mein Studium in Kanada aus der Ferne unterstützten.

Most of all I thank Phil for his encouragement, understanding, and love. The distraction that you, Mr. Darcy and Miss Eliza, and the gang from Hollywood Boulevard offered during the last phase of my degree was most welcome and I thank you all for it.

My research was funded by an operating grant from the Natural Sciences and Engineering Research Council of Canada (NSERC) and a science subvention grant from NSERC and the Department of Fisheries and Oceans, Canada, to Dr. R.E. Scheibling. I was supported by an Izaak Walton Killam Memorial Scholarship, a Dalhousie University Graduate Scholarship, funds from my supervisor, and a Patrick Lett Graduate Student Bursary.

PREFACE

Some of the research described in this thesis has been published in the scientific literature. The references to the publications are as follows:

The research in Chapter 2 is described in:

Meidel, S.K., and R. E. Scheibling. 1998. The annual reproductive cycle of the green sea urchin, *Strongylocentrotus droebachiensis*, in differing habitats in Nova Scotia, Canada. Marine Biology 131: 461-478.

The research in Chapter 3 is also presented in:

Meidel, S.K., and R. E. Scheibling. 1998. Size and age structure of the sea urchin *Strongylocentrotus droebachiensis* in different habitats. In: Echinoderms: San Francisco. Mooi, R., and Telford, M. (eds.). AA Balkema, Rotterdam. pp. 737-742.

Written permissions were obtained from Springer-Verlag (for Chapter 2) and A.A. Balkema (for Chapter 3) to include the material in these publications in my thesis.

Chapter 1: General introduction

Sea urchins are a ubiquitous and common component of marine benthic communities. These important herbivores can be instrumental in structuring the shallow, rocky subtidal zone, often effecting major changes in macroalgal community composition (e.g., Lawrence 1975, Carpenter 1981, Andrew and Choat 1982, Dayton 1985, Hjörleifsson et al. 1995). Along the Atlantic coast of Nova Scotia, large-scale fluctuations in population size of the green sea urchin *Strongylocentrotus droebachiensis* (Müller) have caused dramatic changes in community states over the past three decades (Mann 1977, Wharton and Mann 1981, Miller 1985a, Scheibling 1986, Scheibling et al. submitted). When *S. droebachiensis* are in low abundance, kelp beds (mainly *Laminaria longicruris* and *L. digitata*) flourish in the rocky subtidal zone (Edelstein et al. 1969, Mann 1972). Sea urchins in kelp beds function mainly as detritivores consuming drift algae in crevices and under boulders (Mann 1985). As population size increases, large individuals aggregate along the edge of kelp beds in 'fronts' which destructively graze all macroalgae, leaving barren grounds in their wake (Breen and Mann 1976b, Johnson and Mann 1986, Scheibling et al. submitted). Over the course of several years, sea urchins in grazing fronts can destroy entire kelp beds and transform the subtidal zone to barrens (Breen and Mann 1976b, Mann 1977). The barrens state can persist for decades if sea urchins remain abundant (Himmelman et al. 1983a). Along the Atlantic coast of Nova Scotia, however, sea urchin populations are periodically reduced by disease (Scheibling and Stephensen 1984, Miller 1985a, Miller and Colodey 1985) and the subtidal zone reverts to the kelp bed state as recolonization by macroalgae proceeds (Scheibling 1986, Johnson and Mann 1988).

Drastic reductions in population size of *S. droebachiensis* along the Nova Scotian coast are caused by outbreaks of an amoebic disease (Jones 1985, Jones and Scheibling 1985). Mechanisms leading to sea urchin outbreaks, however, have not been conclusively identified, although sporadic recruitment events (Hart and Scheibling 1988a, Scheibling 1996) or migration (Foreman 1977, Scheibling et al. submitted) may be involved. To assess the role that reproduction may play in the outbreak and general population dynamics of *S. droebachiensis*, we must understand the factors which influence reproductive success in this species. One important factor is the type and amount of food available, which largely determines energy allocation to growth and reproduction. For sea urchins, numerous studies have shown that diet influences gonadal mass (e.g., Moore 1934, Dix 1970, Lang and Mann 1976, Fernandez and Boudouresque 1997), egg quality (e.g., de Jong-Westman et al. 1995b, George 1996), the development of larvae (e.g., Gonzalez et al. 1987, Boidron-Métairon 1988, Hart and Scheibling 1988a, Fenaux et al. 1994), and the maturation of juveniles (Buchanan 1966, Sivertsen and Hopkins 1995).

Several studies have investigated reproductive processes in *S. droebachiensis* throughout the north Pacific and Atlantic areas: e.g., Alaska (Munk 1992), British Columbia (Himmelman 1975), Newfoundland (Keats et al. 1984), Nova Scotia (Lang and Mann 1976), Maine (Wahle and Peckham in press), and Norway (Sivertsen and Hopkins 1995). These studies indicate that variation for example in spawning times, spawning synchrony, or gonad index may result from local and regional differences in diet, temperature and hydrodynamics, or genetic differentiation between populations. This suggests that evaluation of the role that reproduction of *S. droebachiensis* may play in its population dynamics off Nova Scotia requires studies of local populations in the different benthic habitats of the rocky subtidal zone.

In this thesis I investigate the effects of variation in diet quality and quantity on larvae, juveniles and adults of *Strongylocentrotus droebachiensis* in both field and laboratory studies. In Chapter 2, I describe the annual reproductive cycle of sea urchins using gonad index and histological analyses. I relate the observed patterns in the quantity and quality of gonadal material produced by sea urchins in different subpopulations to differences in diet, which I determine through the analysis of gut contents. (I use the term 'subpopulation' for sea urchins in a particular habitat (kelp bed or barrens) or zone (grazing front) in the ecological sense. In my usage, 'subpopulation' does not imply genetic differentiation.) Body size is an important determinant of reproductive output because of its effect on absolute gonad size. In Chapter 3, I document differences in sea urchin size structure among subpopulations and investigate whether these are due to differential growth rates or differences in age structure. I determine the growth rates of juveniles and adults using size-at-age data and again relate observed patterns to patterns of food consumption.

In Chapter 4, I present the results of a laboratory experiment that examines the effects of food ration and feeding regime on reproductive maturation and growth of juvenile sea urchins. Because size (age) at first reproduction and gonad output at each reproductive episode determine the reproductive value of an individual, documentation of the effects of diet on these variables will indicate whether sea urchins in habitats with differing food supplies differ in their life-time reproductive potential. Because adult nutrition may affect larval quality, I conducted a second experiment which compared the importance of parental nutritional condition and larval food ration in determining larval development (Chapter 5). A subset of the adults from the first experiment were used to produce larvae for this experiment.

In Chapter 6, I combine my findings with other published or unpublished data to parameterize models of fertilization kinetics and egg production. Using these models, I estimate the temporal and spatial patterns of zygote production by *S. droebachiensis* in the shallow rocky subtidal zone along the Atlantic coast of Nova Scotia. This theoretical approach allows me to determine the importance of reproductive processes to the population dynamics of *S. droebachiensis* in this region. Finally, in Chapter 7, I briefly review the major findings of my thesis and summarize how this research has contributed to our understanding of the reproductive ecology of *Strongylocentrotus droebachiensis*.

Chapter 2: The annual reproductive cycle of *Strongylocentrotus droebachiensis* in differing habitats in Nova Scotia

INTRODUCTION

Strongylocentrotus droebachiensis has an annual reproductive cycle with a major spawning period (as evidenced by a decline in gonad index) in late winter or early spring (Cocanour and Allen 1967, Himmelman 1978, Falk-Petersen and Lønning 1983, Keats et al. 1984, Munk 1992). Some spawning also has been observed in summer and fall off Newfoundland (Keats et al. 1987). Numerous studies have shown that food quantity and quality strongly influence reproduction of *S. droebachiensis* and other sea urchins (e.g., Lasker and Giese 1954, Ebert 1968, Lawrence 1975, Vadas 1977, Larson et al. 1980). The greater gonad index of *S. droebachiensis* in kelp beds than barrens (Lang and Mann 1976, Wharton 1980b, Johnson and Mann 1982, Keats et al. 1984, Sivertsen and Hopkins 1995) is generally attributed to differences in food availability between these two habitats (Lang and Mann 1976, Sivertsen and Hopkins 1995). However, few investigators have included gut content analysis in their studies and usually only the occurrence of particular food items is recorded (Himmelman and Steele 1971, Chapman 1981, Himmelman and Nédélec 1990). Consequently, there is little quantitative information to compare the amounts and type of food consumed by sea urchins in kelp beds versus barrens.

Wave exposure is another factor which may directly or indirectly influence the reproduction of sea urchins at a site. For example, the supply of drift algae may be greater at wave-exposed sites due to increased wave action which dislodges and

transports plants (Rogers-Bennett et al. 1995). However, Ebert (1968) and Gonor (1973a) found that *Strongylocentrotus purpuratus* at exposed sites had reduced gonad indices compared to those at sheltered sites. Ebert (1968) attributed this difference to a higher cost of repair for broken spines at the exposed site, leaving less energy available for reproduction.

Large-scale fluctuations in population size of *S. droebachiensis* have caused dramatic changes in community state in the shallow, rocky subtidal zone off eastern Canada (Chapter 1). The dynamics of population outbreaks may be influenced by positive feedback mechanisms initiated by the formation of dense grazing fronts along the edge of kelp beds. For example, increased fecundity due to consumption of kelp (Vadas 1977, Larson et al. 1980), or increased fertilization rate due to the proximity of spawning individuals (Pennington 1985), may result in increased larval production. However, few studies have investigated reproductive processes of sea urchins in fronts (Wahle and Peckham submitted), making it difficult to fully evaluate the contribution of individuals in fronts to the total zygote production.

In this study, I compare the reproduction of subpopulations of *Strongylocentrotus droebachiensis* in kelp beds and barrens, and in grazing fronts at the ecotone between these two habitats, at both a wave-exposed and a sheltered site in Nova Scotia. I use both gonad index and histological methods to quantify the reproductive cycle and to examine the effects of habitat and site on maturation and spawning. Also, I compare gut contents of sea urchins in the different habitats and sites to relate differences in reproductive patterns to the quantity and quality of consumed food. Finally, I combine data on reproduction with other population characteristics to examine the relative contribution of sea urchins in kelp beds, grazing fronts, and barrens to the overall larval pool.

MATERIALS AND METHODS

Study sites and sea urchin subpopulations

I studied the reproductive cycle of *Strongylocentrotus droebachiensis* at two sites along the southwestern shore of Nova Scotia: Little Duck Island (44° 22' N, 64° 11' W), a wave-exposed island at the mouth of Mahone Bay, and Mill Cove (44° 35' N, 64° 3' W), a sheltered cove in St. Margaret's Bay. At Little Duck Island, the substratum consisted of basaltic bedrock intersected by ridges and grooves. At Mill Cove, the underlying granitic bedrock was covered with rocks and boulders. At both sites, the study areas were 30x40 m and ranged in depth from 6-9 m.

I compared sea urchins from kelp beds and adjacent barrens, and from grazing fronts at the interface between the two habitats. Kelp beds at both sites consisted of a dense canopy of *Laminaria longicruris* with an understorey of branching (e.g., *Ceramium rubrum*, *Plumaria plumosa*) and foliose algae (e.g., *Chondrus crispus*, *Palmaria palmata*), and articulated coralline algae (*Corallina officinalis*). At Little Duck Island, kelp plants were relatively short with narrow and ruffled blades, a morphology associated with high wave exposure (Gerard and Mann 1979). At Mill Cove, kelp density was lower and the plants were longer, wider, and thinner. Barrens at both sites were dominated by encrusting coralline algae (mainly *Phymatolithon laevigatum*, *Lithothamnion glaciale*) with scattered patches of ephemeral filamentous algae (mainly *Desmarestia viridis*) appearing in summer/fall. Barrens also received input of drift algae (mainly kelp) from the adjacent kelp beds. The grazing front at the interface of the kelp bed and barrens was characterized by kelp stipes (stripped of blades) and articulated corallines, which were the last erect macroalgae to be consumed by the sea urchins.

At both sites, sea urchin density and mean size differed in space and time. In the kelp beds at both sites, sea urchins were sparsely distributed throughout the study

period (mean density: <15 urchins m^{-2}; Scheibling et al. submitted). Sea urchin density was greater in the barrens (mean density: ~80 urchins m^{-2} and ~60 urchins m^{-2} at Little Duck Island and Mill Cove, respectively) and highest in the grazing fronts (100-400 urchins m^{-2} and 100-280 urchins m^{-2}, respectively ; Scheibling and Hennigar 1997, Scheibling et al. 1994, submitted). In October 1993, an outbreak of disease reduced the sea urchin population at Little Duck Island by 87%, but by summer 1995 sea urchin densities had returned to pre-disease levels (Scheibling and Hennigar 1997, Scheibling et al. submitted). Throughout the study period, sea urchins in grazing fronts were much larger (mean test diameter: ~46 mm and ~30 mm at Little Duck Island and Mill Cove, respectively) than those in barrens (~17 mm and ~13 mm, respectively) and kelp beds (~20 mm and ~15 mm, respectively; Scheibling et al. submitted).

Analysis of gonad index

I sampled *Strongylocentrotus droebachiensis* at ~1 month intervals in each habitat at each site between March/April 1994 and August 1995. Additional monthly samples were collected from April 1993 to March 1994 in the barrens and grazing front at Little Duck Island. At each sampling date (except March/April 1995, see below), I collected 8-25 urchins of 35-50 mm test diameter in 10 (grazing front and barrens) or 20 (kelp bed) 0.25 m^2 quadrats. The quadrats were haphazardly placed within a 4x40 m transect in both the kelp bed (~5 m from the offshore edge of the kelp bed) and barrens (10-15 m from the edge), and along 40 m of the approximately 2-m wide grazing front. Sea urchins collected between April 1993 and March 1994 at Little Duck Island were frozen upon return to the laboratory and processed 6-15 months later. Sea urchins collected between March/April 1994 and August 1995 at both sites were kept individually in perforated plastic containers (to enable collection of faeces) in flow-

through aquaria at ambient water temperatures. These sea urchins were processed live within 24-72 h of collection. Total body wet weight and gonad wet weight were measured with an electronic balance (0.01 g accuracy). Gonad index was calculated as [(gonad wet weight / total body wet weight) x 100] to give a percentage. Sex was determined by examining a gonad smear under a compound microscope. Horizontal test diameter was measured with vernier calipers (0.05 mm accuracy).

Temporal patterns in gonad index of female and male sea urchins were compared across habitats (kelp bed, grazing front, barrens) using three-way analysis of variance (ANOVA) with Date (March 1994 to August 1995, when sea urchins were sampled concurrently in all three habitats), Habitat, and Sex as fixed factors. Gonad indices for each sex at the peak of the reproductive cycle were compared between years using one-way ANOVA (grazing front and barrens at Little Duck Island, 1993-1995) or *t*-tests (kelp bed at Little Duck Island, grazing front and barrens at Mill Cove, 1994-1995; a missed sampling interval for the kelp bed at Mill Cove at the peak of the reproductive cycle in 1994 precluded statistical analysis in this habitat). Gonad index at the peak of the reproductive cycle (March/April 1995) and after spawning was completed (June 1995) was compared between sites and sexes, and among habitats (all classified as fixed factors) by three-way ANOVA. I classified Site as a fixed factor because the two study sites were chosen to represent different degrees of exposure to wave action. Raw data were arcsine transformed to remove heterogeneity of variance as indicated by Cochran's *C* test ($p<0.05$). Because sample sizes varied between sites, dates, habitats and sexes, I used Type III sums of squares, and carried out post-hoc comparisons using the GT2-method (Sokal and Rohlf 1994).

To examine changes in gonad index with body size in *Strongylocentrotus droebachiensis* and to confirm that the gonad index of adult sea urchins within the size

range used in my study was independent of test diameter, I sampled 66-75 sea urchins between 14.3-74.9 mm in each habitat at the peak of the reproductive season in 1995 (late March/early April). In *S. droebachiensis*, the development of gonad index with increasing test diameter can be described with a logistic growth model (Munk 1992). I related gonad index to size using the following function:

$$B = \frac{B_0 R}{B_0 + (R - B_0) e^{-g R(D-15)}} \qquad [2.1]$$

where B is gonad index, B_0 is gonad index in immature sea urchins (given a small positive value, 0.1), R is the asymptotic gonad index, g is a constant, and D is test diameter. In all cases the logistic model provided a better fit to my data than a straight-line regression. I used linear regression techniques to analyse the relationship between gonad index (arcsine transformed) and adult body size (35-50 mm) in *S. droebachiensis* at the peak of the reproductive cycle. In ~50% of samples collected at the peak of the reproductive cycle, a few individuals (usually <4 per sex) appeared to have already started to release gametes (i.e., had partly spawned). These sea urchins were excluded from statistical and graphical analysis.

Histological analysis

At each sampling date between June 1994 and May 1995, gonads of 2-12 female and 3-8 (1 in a single case) male sea urchins were prepared using standard histological techniques. Serial cross sections (7 μm) were cut through the centre of a gonad and stained with haematoxylin and eosin. For analysis of reproductive maturation, histological sections were classified according to the six maturity stages used by Byrne (1990) and King et al. (1994): Stage I, recovering; Stage II, growing; Stage III, premature; Stage IV, mature; Stage V, partly spawned; and Stage VI, spent. This

classification scheme is based on changes in the relative abundance of different cell types present in gonads during the maturation process. In samples from February/March and March/April 1995, the ripe gonads of 58 mature sea urchins (27 females, 31 males) disintegrated upon processing and could not be preserved for histological analysis. These sea urchins were classified as mature and included in the analysis of reproductive maturation.

For quantitative analysis of reproductive maturation, histological sections from selected dates from both sites (June, October and December 1994, and February/March, March/April and May 1995) were analysed using light microscopy and a computerised image analysis system (NIH *Image*, Version 1.59; National Institutes of Health, Bethesda, Maryland, USA). Only gonadal acini that fit within the frame size of the image analysis system (719 μm^2) were analysed. For ovaries, the relative areas (expressed as a percentage of total acinal area) of nutritive phagocytes, oocytes, and unoccupied lumen were measured in eight acini, and the absolute areas of oocytes and ova were measured in four acini. Only oocytes sectioned through the nucleolus and ova sectioned through the nucleus were measured. All cells surrounding the germinal cells were classified as nutritive phagocytes. For testes, the relative areas of nutritive phagocytes, spermatocytes, spermatozoa, and unoccupied lumen were measured in eight acini. These measurements were used to quantify the different maturity stages.

The relative areas of nutritive phagocytes (females and males), spermatocytes and spermatozoa, and the absolute areas of oocytes and ova, were compared at each site by two-way ANOVA with Date (June 1994 to May 1995, except for ova: February/March to May 1995 only), and Habitat as fixed factors. For each sea urchin, the relative or absolute area of a cell type was averaged over measurements for all acini and used as a replicate. Relative areas were arcsine transformed to remove heterogeneity

of variance as indicated by Cochran's C test ($p<0.05$). Because sample sizes varied between dates and habitats, I used Type III sums of squares, and carried out post-hoc comparisons using the T' method, which in this case was more conservative than the GT2-method (Sokal and Rohlf 1995).

Analysis of diet

To compare the quality and quantity of food consumed by *Strongylocentrotus droebachiensis* in the different habitats, I analysed the gut contents and faeces of all sea urchins dissected for gonad index analysis at ~1 month intervals between April/May 1994 and August 1995. Food particles were removed from the entire digestive system (pharynx to anus) and added to the faeces collected in the plastic containers prior to dissection. Particles were examined under a dissecting microscope and divided into organic and inorganic material. Organic material consisted of remains of fleshy, filamentous or branching macroalgae, mainly of the genera *Laminaria*, *Chondrus*, *Palmaria*, and *Desmarestia*. Non-organic material consisted of remains of articulated coralline algae, pellets consisting of sediment, and scrapings of encrusting coralline algae. On a few dates, empty zoaria of an epiphytic bryozoan (*Membranipora membranacea*) were present on some kelp particles in gut contents. No other animal remains were observed. The number of food particles in each category, organic or inorganic material, was counted and converted to a percentage. All food particles were then placed in a calibrated vial and allowed to settle for ~1 h, when the total food volume was measured. An index of food quality was calculated as the percentage of the total gut content (plus faeces) that was organic material. An index of food quantity was calculated as the ratio of total food volume to total body volume. Total body volume was estimated from test diameter based on a sample of 96 sea urchins (13-69 mm) collected in

March/April 1995 in all habitats and at both sites. Volume (V) was measured by placing a sea urchin in a water-filled container and weighing the amount of water displaced. The measurement was repeated and the average of the two measurements was log transformed and related to log test diameter (D) by linear regression (r^2=0.998):

$$\ln V = 2.82 \ln D - 7.24 \qquad [2.2]$$

Food quantity and quality were compared by two-way ANOVA with Date (food quantity: June 1994 to August 1995; food quality: April/May 1994 to August 1995) and Habitat as fixed factors. The same sums of squares and post-hoc comparisons were used as for gonad indices.

RESULTS

Spatial and temporal patterns in gonad index

Strongylocentrotus droebachiensis displays a distinct annual cycle of reproduction as indicated by temporal changes in gonad index between 1993 and 1995 (Fig. 2.1). Most spawning occurred in March/April of each year, resulting in a sharp drop in gonad index. In the kelp bed and the grazing front at Mill Cove in 1995, the peak gonad index declined more slowly and spawning may have extended into May. The overall cycle is relatively synchronous across sites and habitats, and also between females and males. At each site, there was a significant interaction between the effects of sampling date and habitat on gonad index (Table 2.1). Post-hoc comparisons (GT2 test) showed that the gonad index in the barrens was significantly lower than in the kelp bed and/or the grazing front on all dates except September 1994 at Little Duck Island, and on all dates except in March and October 1994 at Mill Cove. There also was a significant interaction between the effects of sampling date and sex on gonad index (Table 2.1). At Little Duck Island, females had a significantly higher gonad index than males at the peak

of the gonad index cycle in April 1994 and March 1995, and males had a significantly higher index than females in September 1994. At Mill Cove, females also had a higher gonad index than males in April 1994, and males had a higher index than females in December 1994.

At Little Duck Island, the peak gonad index in the barrens increased significantly from 1993 to 1995 for each sex (females: $F_{2,14}$=5.87, p=0.014; males: $F_{2,11}$=11.39, p=0.002), but there were no significant interannual differences in peak gonad index in either the grazing front (females: $F_{2,19}$=0.90, p=0.422; males: $F_{2,21}$=1.33, p=0.285) or the kelp bed (females: t_9=1.57, p=0.150; males: t_{22}=1.90, p=0.071). At Mill Cove, peak gonad index in the barrens did not differ significantly between 1994 and 1995 (females: t_{11}=0.41, p=0.692; males: t_6=0.98, p=0.365), but the gonad index in the grazing front was significantly higher in 1995 than in 1994 (females: t_{25}=2.28, p=0.031; males: t_{19}=2.90, p=0.009). The peak gonad index immediately prior to spawning in 1995 did not differ significantly between sites ($F_{1,105}$=1.60, p=0.209) but differed consistently between habitats at both sites (i.e., mean gonad index was highest in the kelp bed, lowest in the barrens; $F_{2,105}$=33.34, p<0.001). Gonad index also was consistently higher for females than males ($F_{1,105}$=10.91, p=0.001): there was no significant interaction between site, habitat and sex. The post-spawning gonad index (June 1995) was significantly higher at Little Duck Island than at Mill Cove ($F_{1,84}$=18.06, p<0.001). It was consistently higher in the kelp bed and grazing front than in the barrens at both sites ($F_{2,84}$=41.33, p<0.001), and did not differ significantly between females and males ($F_{1,84}$=1.74, p=0.190): there was no significant interaction between site, habitat and sex.

The relationship between the gonad index and test diameter of *S. droebachiensis* just before spawning (Fig. 2.2) indicates that the development of macroscopic gonads begins at a size of ~15 mm in all habitats at both sites. Gonad index increases rapidly

between 25 and 35 mm and then tends towards an asymptote that is determined by habitat. Linear regression confirmed that there was no relationship between gonad index and test diameter over the size range that I used to monitor the reproductive cycle (35-50 mm) (Table 2.2). There were no signs of reproductive senescence in large individuals up to 75 mm.

Gametogenic cycle

The gametogenic cycles of female and male *S. droebachiensis* were characterized by six maturity stages as illustrated by representative micrographs (Fig. 2.3). In Stage I (recovering) gonadal acini are filled with storage cells (nutritive phagocytes), and small numbers of germinal cells (oocytes in females, spermatocytes in males) are present along the acinal walls (Fig. 2.3a, g). In Stage II (growing), nutritive phagocytes decrease in abundance and are replaced by increasing numbers of oocytes or spermatocytes (Fig. 2.3b, h). In Stage III (premature), nutritive phagocytes further decrease in abundance and the first mature gametes (ova or spermatozoa) begin to accumulate in the lumen (Fig. 2.3c, i). In Stage IV (mature), most of the lumen is occupied by mature gametes, and nutritive phagocytes are reduced to a thin layer along the acinal wall (Fig. 2.3d, j). In Stage V (partly spawned), the lumen is emptied as mature gametes are shed but not yet replaced to any great extent by nutritive phagocytes (Fig. 2.3e, k). In Stage VI (spent), some relict oocytes/ova or spermatozoa may be present in the lumen, which is accumulating a growing layer of nutritive phagocytes (Fig. 2.3f, l).

The gametogenic cycle of *S. droebachiensis* was approximately synchronous between sites and across habitats for both males and females, although individuals could be found in two or three different maturity stages on most dates (Figs. 2.4, 2.5). After

spawning in spring, females remained in the recovering stage (Stage I) for 2-4 months before moving into the growing stage (Stage II) during the summer (Fig. 2.4). By late summer or early fall, most females had entered the premature stage (Stage III) where they remained until late winter or early spring when they became fully mature (Stage IV). Females proceeded rapidly through the partly spawned (Stage V) and spent stages (Stage VI) and started a new gametogenic cycle a few weeks after spawning. At Mill Cove, one partly spawned female was found in September in the kelp bed (Fig. 2.3e). Males of *S. droebachiensis* showed a similar pattern of maturation as females, although the periodicity was less pronounced (Fig. 2.5). After spawning, most males entered the recovering and growing stages in early or mid-summer, although up to 30% of males in some habitats (Little Duck Island, barrens; Mill Cove, kelp bed) remained in the spent stage until late summer. At Little Duck Island, most males entered the premature stage in late fall while at Mill Cove ~25% of males were still in the growing stage in February. Most males were fully mature in late winter or early spring, and proceeded through the partly spawned and spent stages within 1-2 months of spawning before starting a new gametogenic cycle. At Mill Cove, one mature male was found in October in both the grazing front and the barrens (Fig. 2.3j), and one partly spawned male was found in November in the grazing front (Fig. 2.3k).

Changes in gonadal microstructure during maturation

The proportion (by cross-sectional area of a gonadal acinus) of nutritive phagocytes in ovaries of females of *Strongylocentrotus droebachiensis* showed a distinct annual cycle that was synchronous across sites and habitats (Fig. 2.6). After the major spawning period in March/April, the proportion of nutritive phagocytes increased rapidly within two months. As gametogenesis proceeded, the proportion of nutritive

phagocytes progressively decreased to a minimum just prior to the next major spawning period. The proportion of nutritive phagocytes in the ovaries differed significantly between dates at both sites (Table 2.3), and it was significantly lower in the kelp bed than in the grazing front at Little Duck Island (T' test). Mean oocyte area increased throughout the maturation cycle and reached a maximum just prior to spawning, when it decreased sharply as large oocytes matured into ova and newly produced oocytes were small (Fig. 2.6). At Little Duck Island, mean oocyte area differed significantly between dates but not between habitats (Table 2.3). At Mill Cove, there was a significant interaction between date and habitat: mean oocyte area was significantly lower in the grazing front than in the barrens in February and the kelp bed in March. While oocytes were present at all times, ova first appeared in late winter and were lost at spawning (Fig. 2.6). There were no significant differences in mean ova area between months or habitats at either site (Table 2.3). The relative abundance of ova, and the proportions of oocytes, nutritive phagocytes, and unoccupied lumen were used to quantify the maturity stages of females (Table 2.4).

Males of *Strongylocentrotus droebachiensis* showed the same temporal pattern in the proportion of nutritive phagocytes in the gonads as females (Fig. 2.7). The proportion of nutritive phagocytes increased rapidly after spawning and then progressively decreased until the next major spawning period. The proportions of spermatocytes and spermatozoa (Fig. 2.7) showed a reciprocal pattern of abundance relative to nutritive phagocytes. After spawning, the proportion of spermatocytes increased to a maximum in early winter and remained at that level until the next spawning. The proportion of spermatozoa dropped sharply after spawning and remained low during the summer, increasing in fall and winter to a maximum at the peak of the reproductive cycle. At Little Duck Island, there was a significant interaction between the

effects of date and habitat on the proportions of all three cell types in the testes (Table 2.5). The proportion of nutritive phagocytes was significantly higher and the proportion of spermatozoa significantly lower in the barrens than in the kelp bed and/or grazing front in October 1994 and May 1995. The proportion of spermatocytes also was significantly lower in the barrens than in the kelp bed and grazing front in May 1995. At Mill Cove, there was a significant effect of date on the proportions of both nutritive phagocytes and spermatocytes but no significant effect of habitat (Table 2.5). Also at Mill Cove, there was a significant interaction between the effects of date and habitat on the proportion of spermatozoa which was significantly higher in the barrens than in the grazing front in February 1995. The proportions of spermatocytes, spermatozoa, nutritive phagocytes, and unoccupied lumen were used to quantify the maturity stages of males (Table 2.6).

Sex ratio

Sex ratios of *Strongylocentrotus droebachiensis* did not deviate significantly from 1:1 (χ^2-test, $p>0.05$) in any habitat at either site with the exception of the kelp bed at Little Duck Island, where males were more abundant than females (148 males, 115 females; $\chi^2=4.141$, $p<0.05$). Samples in which >10% of urchins could not be sexed were excluded from analysis. Three hermaphrodites were observed at Mill Cove (one from each habitat), which represented 0.35% of sea urchins sampled at that site (n=862) and 0.15% of the total sampled at both sites (n=1968).

Gut content analysis

The food quantity index of *Strongylocentrotus droebachiensis* was temporally variable in all habitats at both sites but tended to be lowest in late summer and early fall

(Fig. 2.8). At Little Duck Island the index increased in the kelp bed and grazing front after spawning (March/April) in 1995. At both sites, there was a significant interaction between the effects of date and habitat on the food quantity index (Table 2.7). At Little Duck Island, the index was significantly lower in the barrens than in the kelp bed and/or the grazing front in fall 1994 and spring 1995 (5 out of 11 dates; GT2 test) and significantly lower in the kelp bed than in the grazing front and/or barrens in late summer and fall 1994 and June 1995 (6 out of 11 dates). At Mill Cove, the food quantity index was significantly lower in the barrens than in the kelp bed and/or grazing front in June and December 1994, and in late winter/early spring 1995 (5 out of 12 dates).

The food quality index of *Strongylocentrotus droebachiensis* (Fig. 2.8) was consistently high in the kelp bed and grazing front and more variable but generally lower in the barrens at both sites. As with the food quantity index, there also was a significant interaction between the effects of date and habitat on the food quality index (Table 2.7). At Little Duck Island, the food quality index was significantly lower in the barrens than in the kelp bed and/or grazing front in spring and fall 1994, and spring and summer 1995 (8 out of 13 dates). At Mill Cove, this was the case in summer and winter 1994 and throughout 1995 (9 out of 13 dates).

Table 2.1. Three-way ANOVA of the effects of Date (D), Habitat (H), and Sex (S) on gonad index and GT2 post-hoc comparisons of the simple effects of Habitat (sexes pooled) and Sex (habitats pooled) at each date at Little Duck Island and Mill Cove. [Date: Mar/Apr 1994 to Aug 1995. Habitat: kelp bed (KB), grazing front (GF), barrens (BG). Sex: female (f), male (m). NS, not significant; nd, no data; $^{*}p<0.05$; $^{***}p<0.001$]

Source	Little Duck Island				Mill Cove			
ANOVA	*df*	MS	*F*	*p*	*df*	MS	*F*	*p*
D	12	609.46	76.66	<0.001***	14	542.42	45.61	<0.001***
H	2	1069.63	134.55	<0.001***	2	1314.60	110.53	<0.001***
S	1	17.06	2.15	0.144	1	0.52	0.04	0.834
D x H	24	29.73	3.74	<0.001***	28	49.22	4.14	<0.001***
D x S	12	34.27	4.31	<0.001***	14	21.09	1.77	0.039*
H x S	2	2.66	0.33	0.716	2	4.59	0.39	0.680
D x H x S	24	7.43	0.94	0.553	28	14.42	1.21	0.209
Error	571	7.95			64	11.89		

GT2 test	Habitat	Sex	Habitat	Sex
Mar 1994	nd	nd	NS	NS
Apr 1994	KB>GF>BG	f>m	GF>BG	f>m
May 1994	KB, GF>BG	NS	KB, GF>BG	NS
Jun 1994	GF>BG	NS	KB>GF>BG	NS
Jul/Aug 1994	KB>BG	NS	KB, GF>BG	NS
Sep 1994	NS	f<m	KB, GF>BG	NS
Oct 1994	KB>GF, BG	NS	NS	NS
Nov 1994	KB>GF, BG	NS	KB, GF>BG	NS
Dec 1994	KB>GF, BG	NS	KB, GF>BG	f<m
Jan 1995	nd	nd	KB>GF, BG	NS
Feb/Mar 1995	KB, GF>BG	f>m	KB, GF>BG	NS
Mar/Apr 1995	KB, GF>BG	NS	KB>GF>BG	NS
May 1995	KB, GF>BG	NS	KB, GF>BG	NS
Jun 1995	KB, GF>BG	NS	KB, GF>BG	NS
Aug 1995	KB, GF>BG	NS	KB>GF>BG	NS

Table 2.2. Results of linear regression analysis of gonad index (arcsine transformed) on test diameter (34.5-52.2 mm). [n, sample size; r^2, coefficient of determination; p, probability]

Site, Habitat	Size range (mm)	n	r^2	p
Little Duck Island				
Kelp bed	35.2 - 49.8	17	0.152	0.122
Grazing front	34.5 - 50.9	13	0.035	0.541
Barrens	35.0 - 50.2	15	0.104	0.240
Mill Cove				
Kelp bed	34.7 - 52.2	28	0.033	0.650
Grazing front	35.0 - 50.3	24	0.008	0.685
Barrens	34.7 - 50.0	20	0.057	0.310

Table 2.3. Two-way ANOVA of the effects of Date (D) and Habitat (H) on proportions of nutritive phagocytes, and absolute areas of oocytes and ova of females at Little Duck Island and Mill Cove. [Date: Jun, Oct, Dec 1994, and Feb/Mar, Mar/Apr and May 1995. Habitat: kelp bed, grazing front, barrens. $^{*}p<0.05$; $^{***}p<0.001$]

Source	Little Duck Island				Mill Cove			
	df	MS	*F*	*p*	*df*	MS	*F*	*p*
Nutritive phagocytes								
D	5	3895.49	78.63	<0.001***	5	3155.45	26.43	<0.001***
H	2	359.02	7.25	0.001**	2	103.97	0.87	0.422
D x H	10	87.17	1.76	0.081	10	139.61	1.17	0.323
Error	84	49.54			85	119.37		
Oocytes								
D	5	111021228	57.02	<0.001***	5	88994232	36.29	<0.001***
H	2	317298	0.16	0.850	2	10172671	4.15	0.019*
D x H	10	2319244	1.19	0.309	10	5103536	2.08	0.035*
Error	83	1946993			85	2452460		
Ova								
D	2	3703055	2.38	0.123	1	1916321	0.45	0.510
H	2	5563400	3.57	0.051	2	11946850	2.78	0.081
D x H	3	1980219	1.27	0.316	2	519675	0.12	0.887
Error	17	1559115			25	4295489		

Table 2.4. Stages of the ovarian cycle. Data are qualitative records of abundance of ova, and relative areas (as the percentage of the cross-sectional area of ovarian acini) of oocytes, nutritive phagocytes, and unoccupied lumen. Note: because large numbers of ova oozed from ripe gonads upon processing, it was not possible to measure their proportion in histological sections; their relative abundance was approximated instead.

Stage	Ova abundance	Oocytes (%)	Nutritive phagocytes (%)	Lumen (%)
I. Recovering	none	< 15	> 75	< 15
II. Growing	none	15-40	40-75	< 5
III. Premature	few	> 40	10-40	< 5
IV. Mature	very many	< 5	< 10	< 5
V. Partly spawned	some	< 5	10-30	40-70
VI. Spent	few relict	< 5	> 30	< 40

Table 2.5. Two-way ANOVA of the effects of Date (D) and Habitat (H) on proportions of nutritive phagocytes, spermatocytes and spermatozoa of males at Little Duck Island and Mill Cove. [Date: Jun, Oct, Dec 1994, and Feb/Mar, Mar/Apr and May 1995. Habitat: kelp bed, grazing front, barrens. $^{*}p<0.05$; $^{**}p<0.01$; $^{***}p<0.001$]

Source	Little Duck Island				Mill Cove			
	df	MS	*F*	*p*	*df*	MS	*F*	*p*
Nutritive phagocytes								
D	5	4465.30	57.86	<0.001***	5	2240.41	9.45	<0.001***
H	2	439.31	5.69	0.005**	2	14.40	0.06	0.941
D x H	10	298.38	3.87	<0.001***	10	297.35	1.25	0.275
Error	61	77.17			66	237.12		
Spermatocytes								
D	5	1125.23	30.12	<0.001***	5	1184.90	9.21	<0.001***
H	2	547.54	14.66	<0.001***	2	322.25	2.50	0.090
D x H	10	341.29	9.14	<0.001***	10	227.23	1.77	0.085
Error	61	37.35			66	128.69		
Spermatozoa								
D	5	3143.93	33.98	<0.001***	5	1268.62	4.86	<0.001***
H	2	468.94	5.07	0.009**	2	87.85	0.34	0.716
D x H	10	220.11	2.38	0.019*	10	600.32	2.30	0.022*
Error	61	92.53			66	261.06		

Table 2.6. Stages of the testicular cycle. Data are relative areas (as the percentage of the cross-sectional area of testicular acini) of spermatocytes, spermatozoa, nutritive phagocytes, and unoccupied lumen.

Stage	Spermatocytes (%)	Spermatozoa (%)	Nutritive phagocytes (%)	Lumen (%)
I. Recovering	< 10	0	> 80	< 10
II. Growing	10-40	0	40-80	< 5
III. Premature	> 40	> 15	10-40	< 5
IV. Mature	< 5	> 70	< 10	0
V. Partly spawned	< 5	> 10	10-30	> 20
VI. Spent	< 5	< 10	> 30	< 20

Table 2.7. Two-way ANOVA of the effects of Date (D) and Habitat (H) on food quantity and quality index, and GT2 post-hoc comparisons of the simple effects of Habitat at each date at Little Duck Island and Mill Cove. [Date: food quantity, Jun 1994 to Aug 1995; food quality, Apr/May 1994 to Aug 1995. Habitat: kelp bed (KB), grazing front (GF), barrens (BG). NS, not significant; nd, no data; $^{***}p<0.001$]

Source	Little Duck Island				Mill Cove			
	df	MS	*F*	*p*	*df*	MS	*F*	*p*
Food quantity index								
D	10	601.93	29.88	<0.001***	11	1766.08	69.86	<0.001***
H	2	816.91	40.55	<0.001***	2	263.60	10.43	<0.001***
D x H	20	104.84	5.20	<0.001***	22	100.80	3.99	<0.001***
Error	596	20.15			668	25.28		
Food quality index								
D	12	3362.22	10.84	<0.001***	12	3607.42	7.43	<0.001***
H	2	18332.85	59.11	<0.001***	2	85209.63	175.55	<0.001***
D x H	24	1734.20	5.59	<0.001***	24	4717.86	9.72	<0.001***
Error	685	310.12			710	485.37		

GT2 test	Food quantity	Food quality	Food quantity	Food quality
Apr 1994	nd	NS	nd	nd
May 1994	nd	KB>BG	nd	NS
Jun 1994	NS	KB>GF, BG	KB>BG	KB>GF>BG
Jul/Aug 1994	GF, BG>KB	NS	NS	KB>GF, BG
Sep 1994	GF, BG>KB	BG>GF	NS	NS
Oct 1994	GF>KB	KB, GF>BG	NS	NS
Nov 1994	GF>KB,BG	NS	NS	NS
Dec 1994	GF>BG>KB	NS	KB, GF>BG	KB>GF>BG
Jan 1995	nd	nd	NS	KB>GF>BG

Table 2.7 cont.

	Little Duck Island		Mill Cove	
GT2 test	Food quantity	Food quality	Food quantity	Food quality
Feb/Mar 1995	NS	KB, GF>BG	GF>BG	KB, GF>BG
Mar/Apr 1995	KB, GF>BG	KB>GF>BG	KB, GF>BG	KB, GF>BG
May 1995	GF>BG	KB>BG	KB, GF>BG	KB, GF>BG
Jun 1995	GF>KB>BG	KB, GF>BG	NS	KB>GF>BG
Aug 1995	NS	KB, GF>BG	BG>KB	KB, GF>BG

Fig. 2.1. Mean gonad index (percentage of total body wet weight, +SD) for female, male or unsexed sea urchins (35-50 mm test diameter) at Little Duck Island and Mill Cove between April 1993 and August 1995 in the kelp bed, the grazing front and the barrens. Means are based on 2-17 sea urchins.

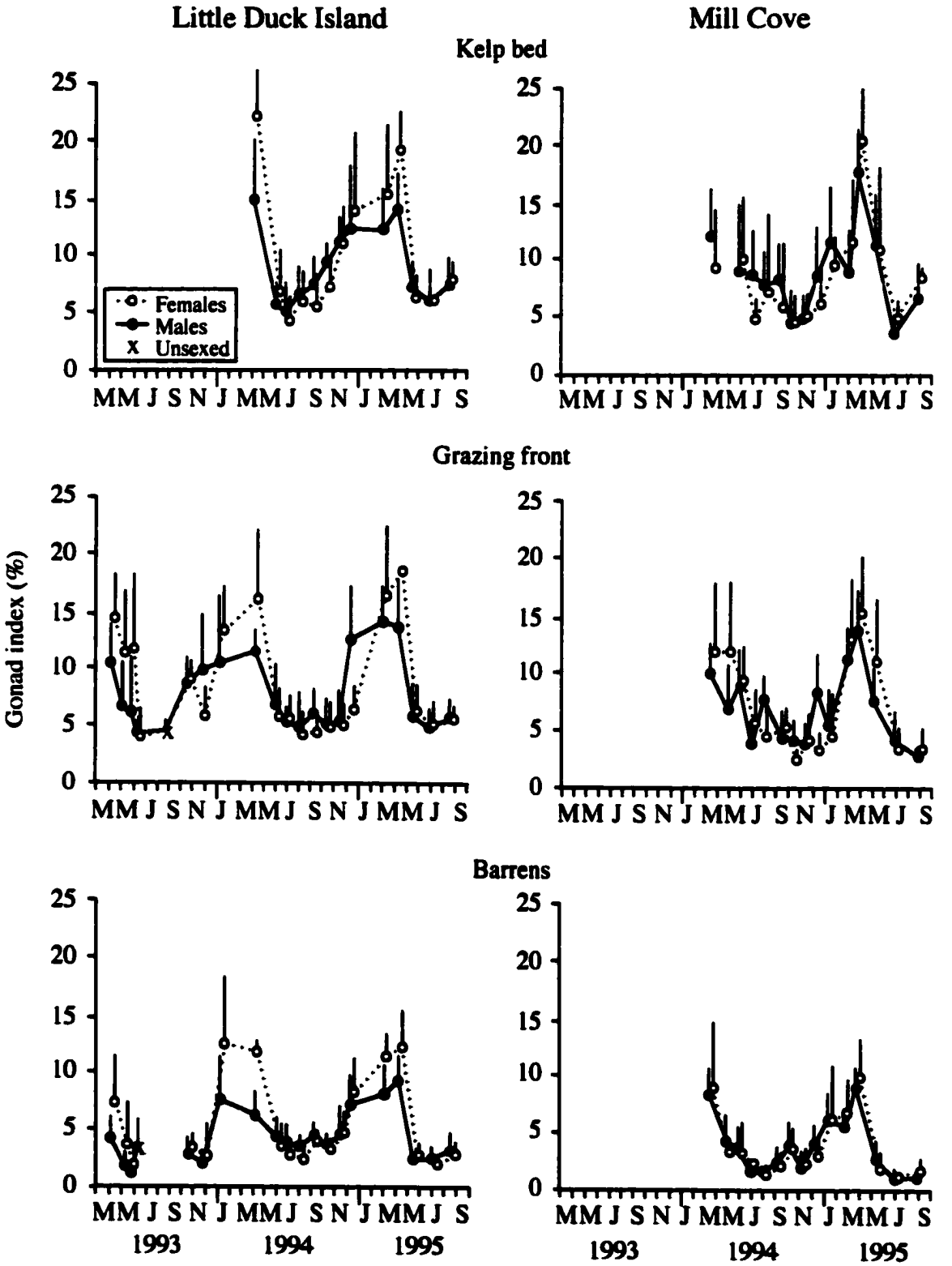
Little Duck Island
Mill Cove
Kelp bed
Grazing front
Barrens
Gonad index (%)
Females
Males
Unsexed
0
5
10
15
20
25
MM J S N J MM J S N J MM J S
1993
1994
1995

Figure 2.1

Fig. 2.2. Relationship between gonad index and test diameter (14.3-74.9 mm) in March (Mill Cove) and April (Little Duck Island) 1995 in the kelp bed, the grazing front, and the barrens. The plotted line represents the fit of Eq. 2.1 to each data set. Parameter values for R (asymptotic gonad index), and g (a constant) are given for each relationship. B^0 (gonad index in juveniles) equals 0.1 in all cases. (n, sample size; r^2, coefficient of determination).

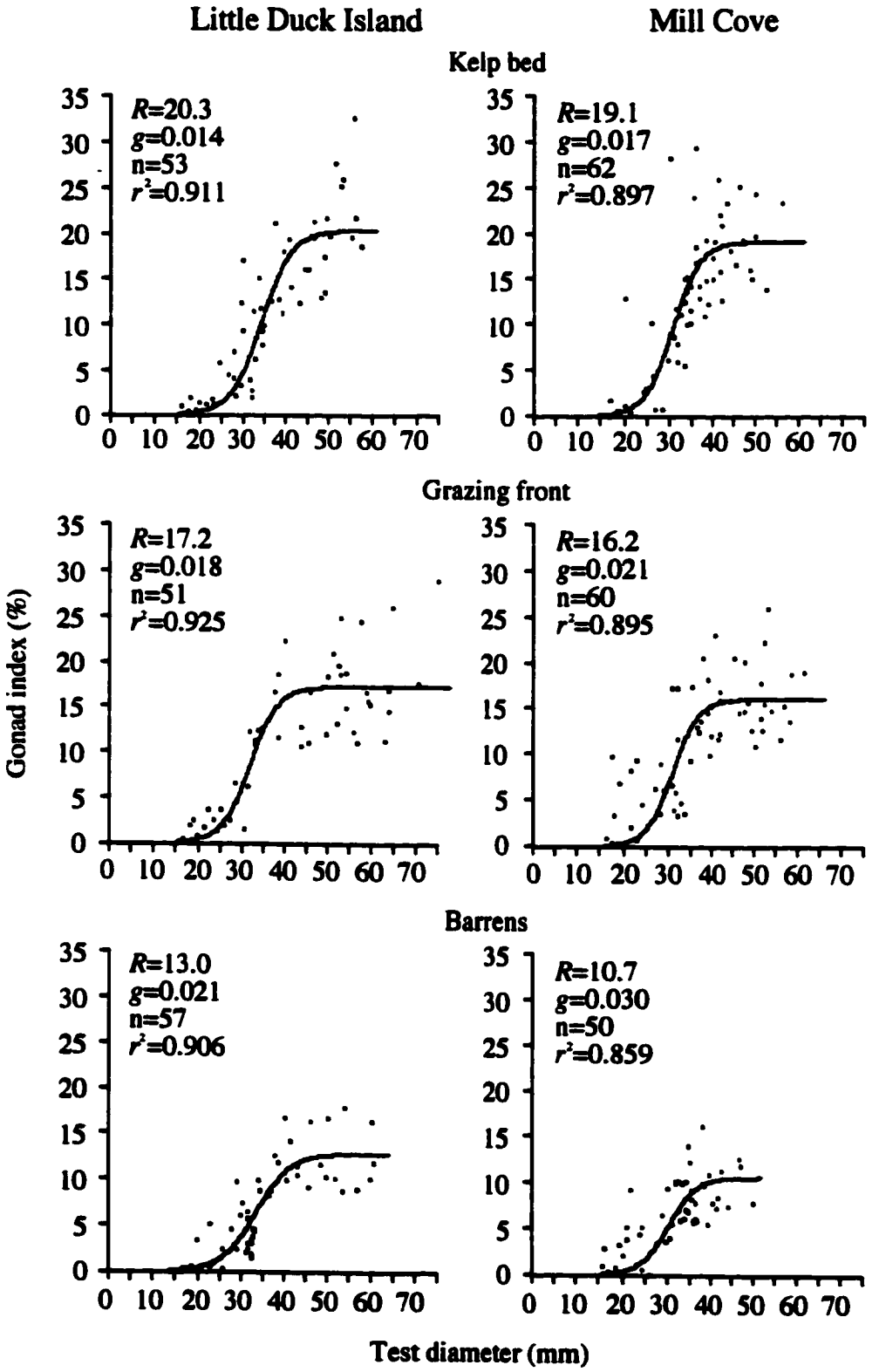
Little Duck Island
Mill Cove
Kelp bed
Grazing front
Barrens
R=20.3
g=0.014
n=53
r²=0.911
R=19.1
g=0.017
n=62
r²=0.897
R=17.2
g=0.018
n=51
r²=0.925
R=16.2
g=0.021
n=60
r²=0.895
R=13.0
g=0.021
n=57
r²=0.906
R=10.7
g=0.030
n=50
r²=0.859
Gonad index (%)
Test diameter (mm)
0 5 10 15 20 25 30 35
0 10 20 30 40 50 60 70

Figure 2.2

Fig. 2.3. Histology of ovaries (a-f) and testes (g-l). (a) Stage I: recovering ovary with nutritive phagocytes (NP) filling lumen; few small oocytes (Oc) along acinal wall. (b) Stage II: growing ovary with more abundant and larger oocytes along acinal wall. (c) Stage III: premature ovary with many oocytes accumulating in lumen; nutritive phagocyte layer reduced. (d) Stage IV: mature ovary filled with ova (O); nutritive phagocytes are reduced to thin layer along acinal wall (Nu nucleus. (e) Stage V: partly spawned ovary with spaces vacated by spawned ova. (f) Stage VI: spent ovary with relict ova and few new oocytes; nutritive phagocyte layer increasing in thickness. (g) Stage I: recovering testis with nutritive phagocytes (NP) filling lumen; thin layer of spermatocytes (Sc) along acinal wall. (h) Stage II: growing testis with spermatocyte layer increasing in thickness. (i) Stage III: premature testis with spermatozoa (Sz) accumulating in lumen; nutritive phagocyte layer reduced. (j) Stage IV: mature testis filled with spermatozoa; nutritive phagocytes are reduced to thin layer along acinal wall. (k) Stage V: partly spawned testis with spaces vacated by spawned spermatozoa (L lumen). (l) Stage VI: spent testis with nutritive phagocytes almost filling lumen; scattered spermatocytes along acinal wall. (Scale bars: 100 μm)

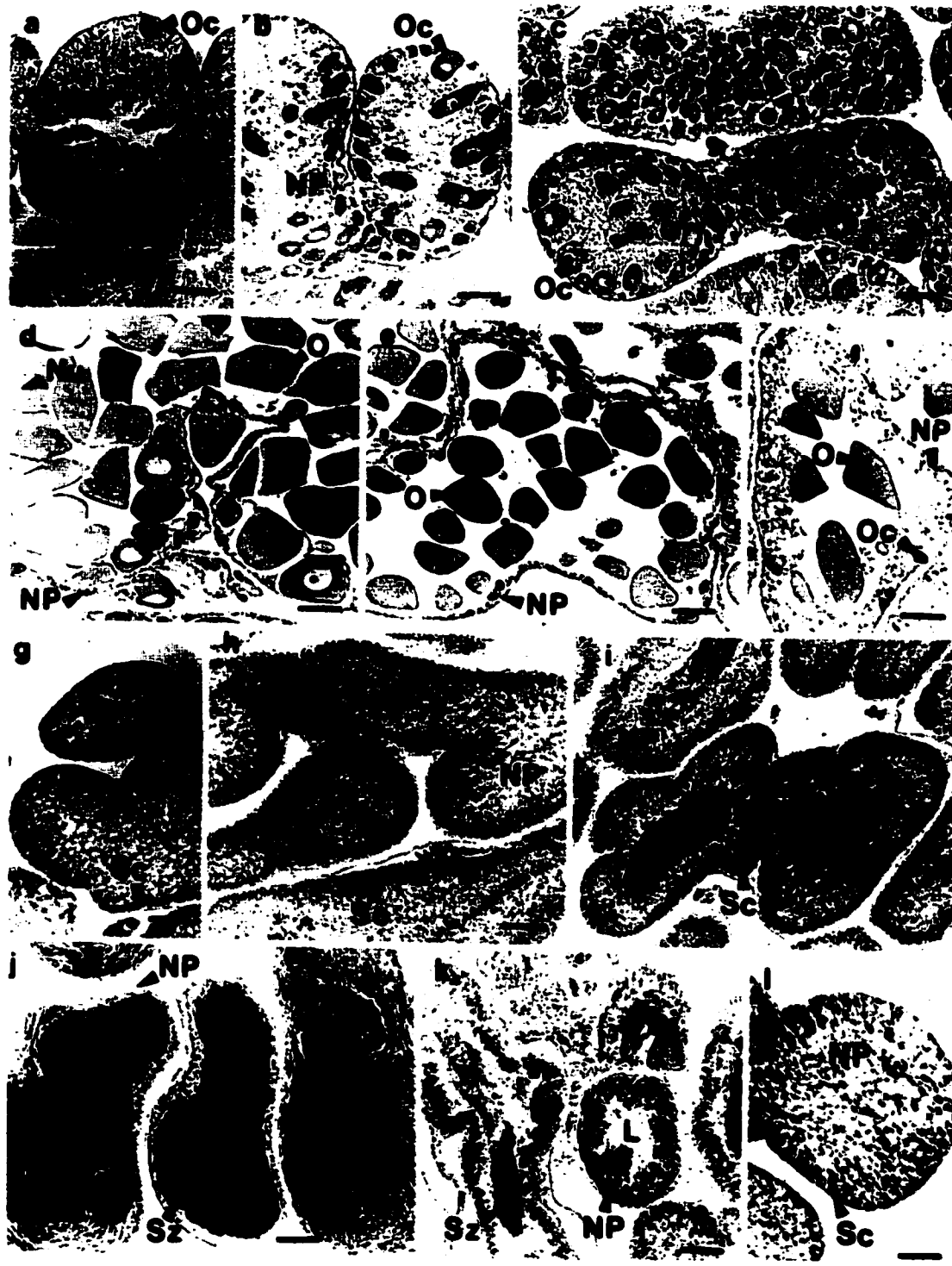

Figure 2.3

Fig. 2.4. Frequencies (%) of females in Stages I-VI of the reproductive cycle at Little Duck Island and Mill Cove between June 1994 and June 1995 in the kelp bed, the grazing front, and the barrens. Stage I: recovering, Stage II: growing, Stage III: premature, Stage IV: mature, Stage V: partly spawned, and Stage VI: spent. Numbers above bars indicate sample size.

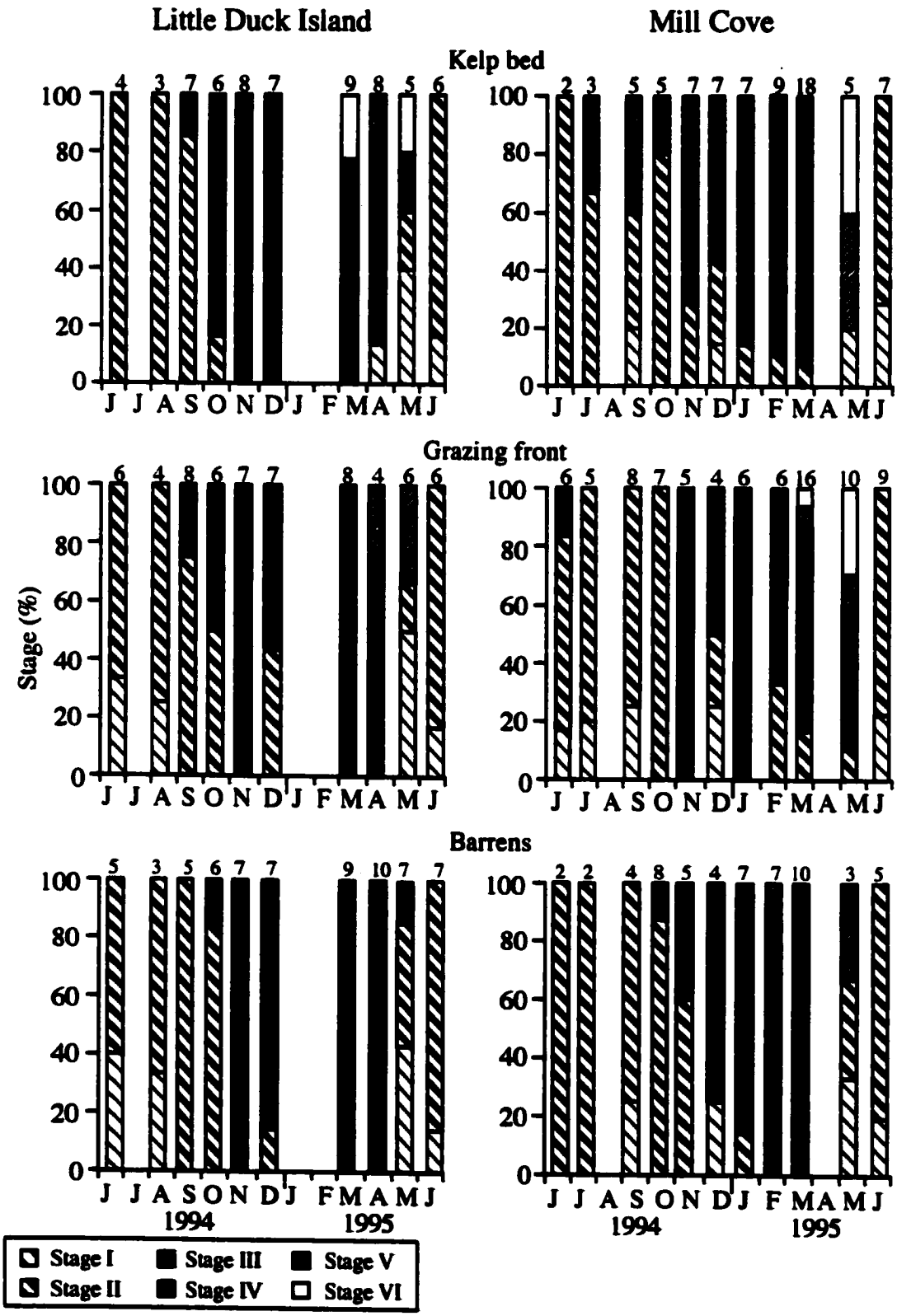
Little Duck Island
Mill Cove
Kelp bed
Grazing front
Barrens
Stage (%)
J J A S O N D J F M A M J
1994
1995
Stage I
Stage II
Stage III
Stage IV
Stage V
Stage VI

Figure 2.4

Fig. 2.5. Frequencies (%) of males in Stages I-VI of the reproductive cycle at Little Duck Island and Mill Cove between June 1994 and June 1995 in the kelp bed, the grazing front, and the barrens. For stage description see Fig. 2.4. Numbers above bars indicate sample size.

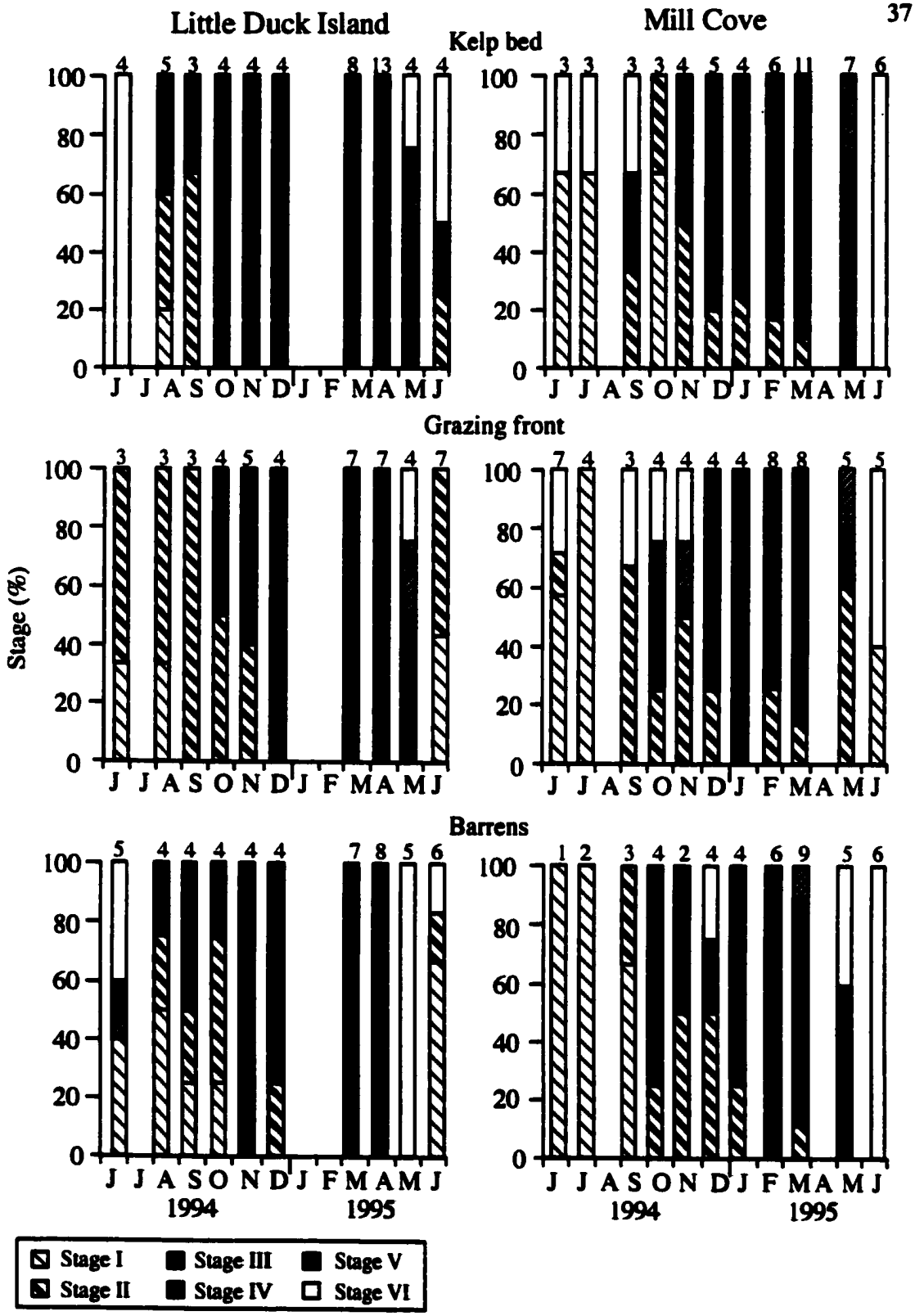
Little Duck Island
Mill Cove
Kelp bed
Grazing front
Barrens
Stage (%)
J J A S O N D J F M A M J
1994
1995
Stage I
Stage II
Stage III
Stage IV
Stage V
Stage VI

Figure 2.5

Fig. 2.6. Mean (+SD) relative area (percentage of cross-sectional area of gonadal acini) of nutritive phagocytes, and mean (+SD) absolute areas of oocytes and ova of female sea urchins in the kelp bed, grazing front and barrens at Little Duck Island and Mill Cove between June 1994 and May 1995. Means are based on 2-12 sea urchins.

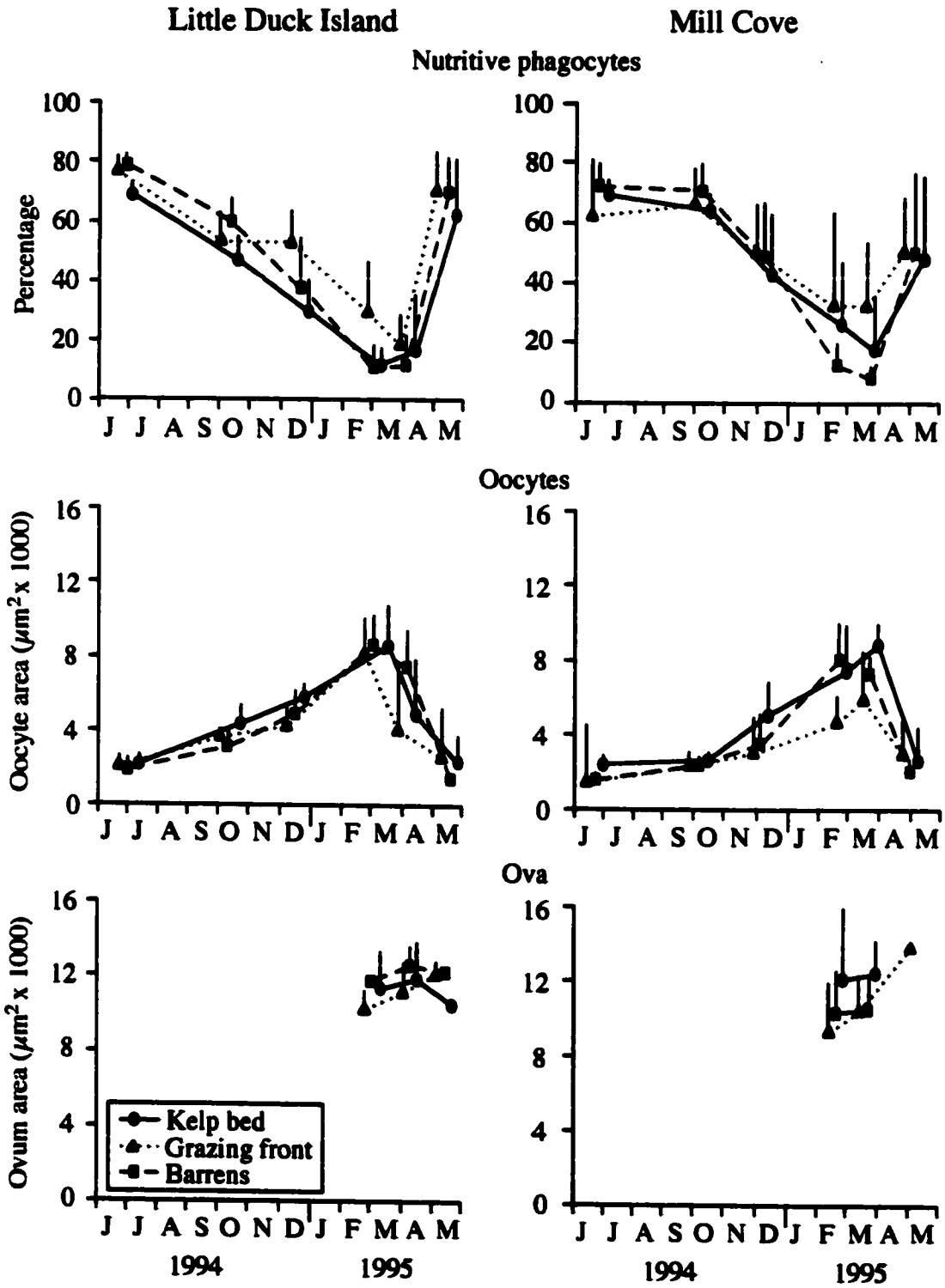
Little Duck Island
Mill Cove
Nutritive phagocytes
Percentage
Oocytes
Oocyte area (μm² x 1000)
Ova
Ovum area (μm² x 1000)
Kelp bed
Grazing front
Barrens
J J A S O N D J F M A M
1994
1995

Figure 2.6

Fig. 2.7. Mean (+SD) relative abundance (percentage of cross-sectional area of gonadal acini) of nutritive phagocytes, spermatocytes, and spermatozoa of male sea urchins in the kelp bed, grazing front and barrens at Little Duck Island and Mill Cove between June 1994 and May 1995. Means are based on 3-8 sea urchins.

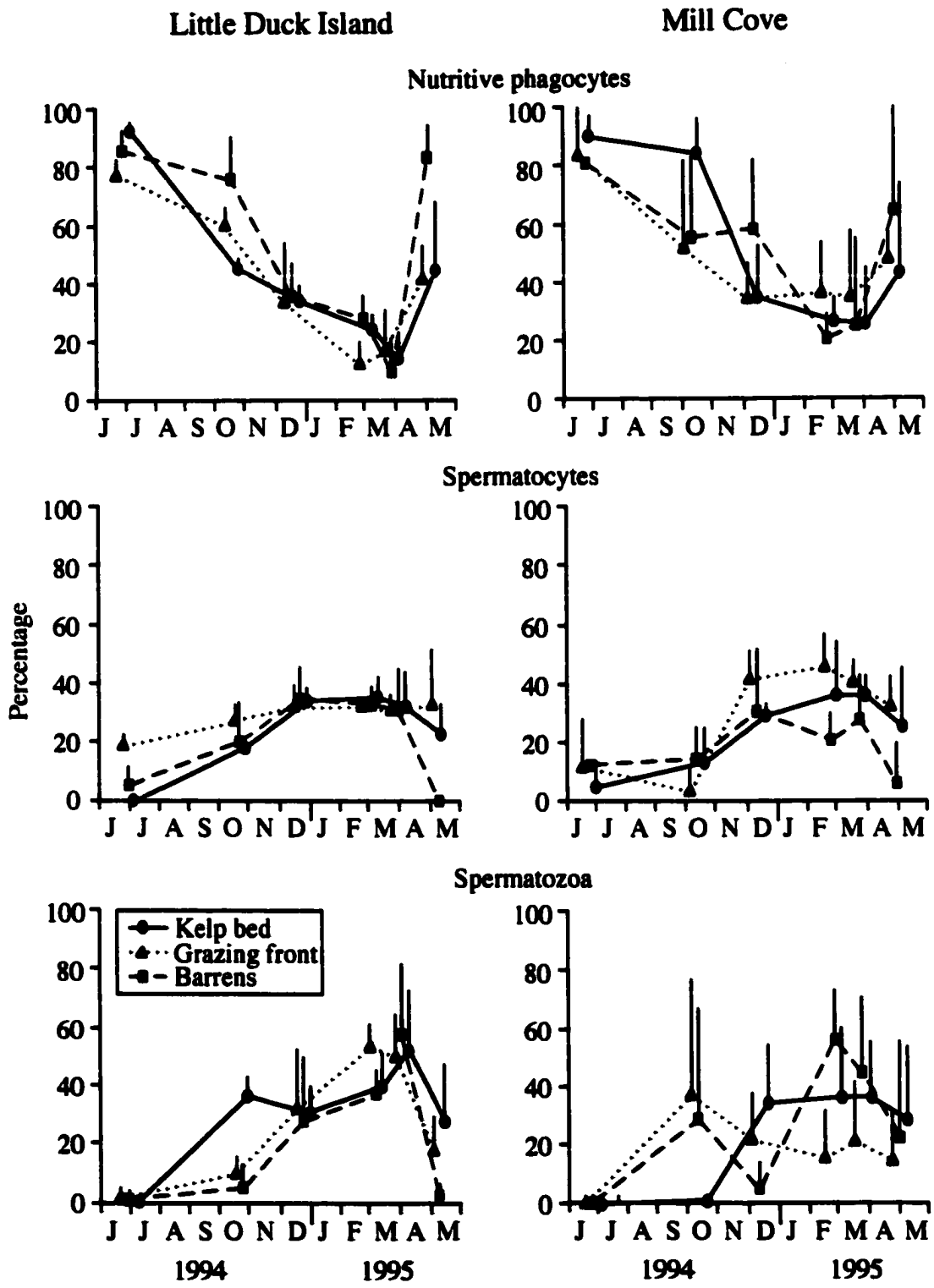
Little Duck Island
Mill Cove
Nutritive phagocytes
Spermatocytes
Spermatozoa
Percentage
100
80
60
40
20
0
J J A S O N D J F M A M
1994
1995
Kelp bed
Grazing front
Barrens

Figure 2.7

Fig. 2.8. Indices of food quantity and food quality (mean ±SD) at Little Duck Island and Mill Cove between April (quality) or June 1994 (quantity) and August 1995 in the kelp bed, grazing front and barrens. Food quantity is expressed as food volume (percentage of total body volume) and food quality as organic material (percentage of total gut content). Means are based on 5-34 sea urchins.

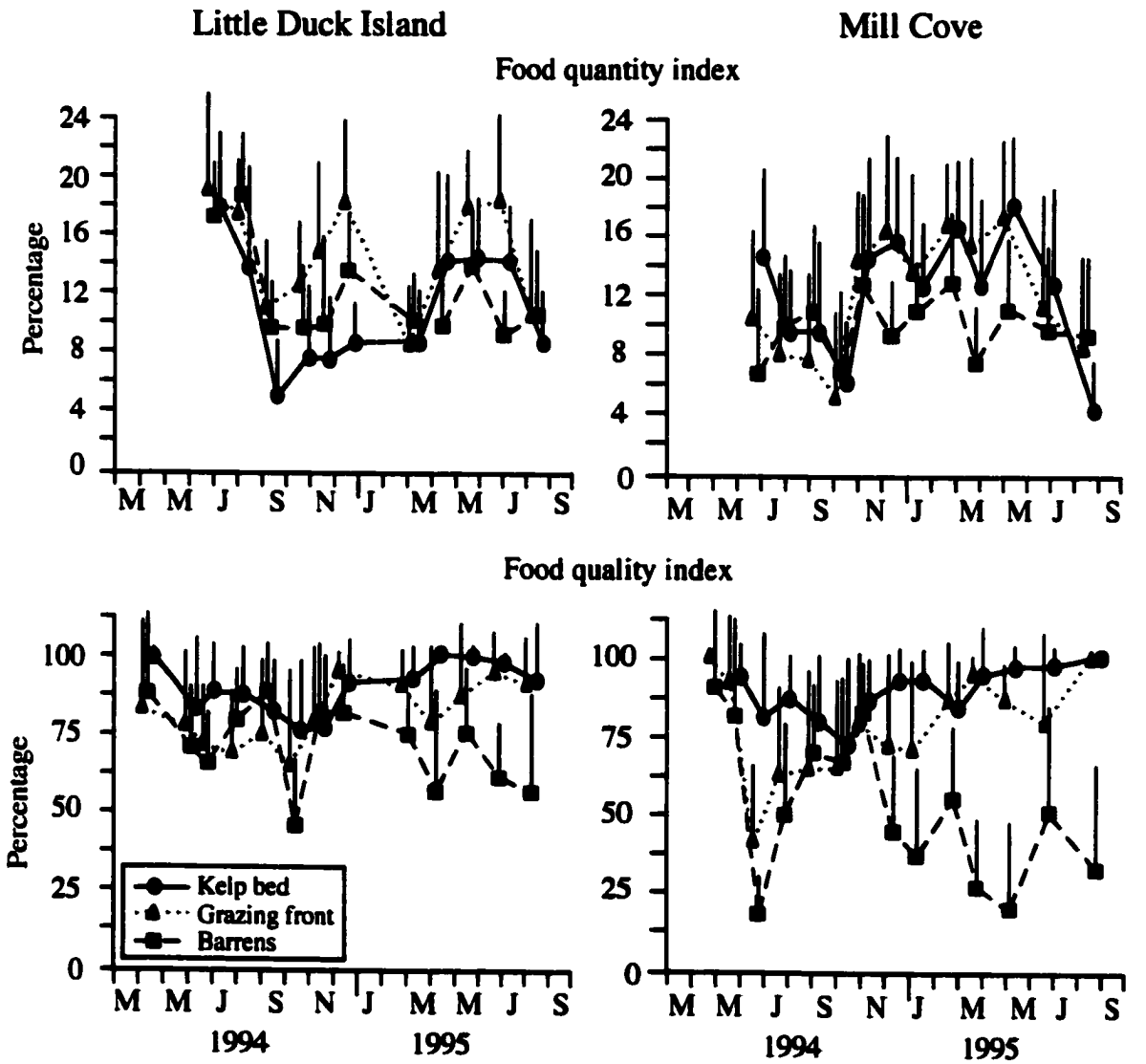
Little Duck Island
Mill Cove
Food quantity index
Percentage
24
20
16
12
8
4
0
M M J S N J M M J S
Food quality index
100
75
50
25
0
Kelp bed
Grazing front
Barrens
1994
1995

Figure 2.8

DISCUSSION

Reproductive cycle

Strongylocentrotus droebachiensis on the Atlantic coast of Nova Scotia exhibits a distinct annual reproductive cycle with a major spawning period in early spring. The cycle was relatively synchronous between habitats differing in food quality and quantity, and between sites differing in wave exposure. Previous studies have shown a similar cycle of gonad index for *S. droebachiensis* in Maine (Cocanour and Allen 1967), Newfoundland (Himmelman 1978, Keats et al. 1984), and Norway (Falk-Petersen and Lønning 1983). Histological analysis also indicated a similar progression of non-gametic and gametic cells as previously described for females of *S. droebachiensis* (Falk-Petersen and Lønning 1983) and for both sexes of other strongylocentrotids (e.g., Fuji 1960a, Chatlynne 1969, Gonor 1973a, b). Nutritive phagocytes were most abundant at the beginning of the reproductive cycle and were subsequently replaced by increasing numbers of germinal and gametic cells (oocytes and ova in females, spermatocytes and spermatozoa in males).

The general synchrony of reproduction in all habitats suggests that the annual reproductive cycle is controlled by factors other than food, possibly temperature and/or photoperiod (e.g., Gonor 1973a). Individual sea urchins, however, usually occurred in two or three gametogenic stages at any one time, with the greatest variability present during the spawning period. Such variation, which also has been documented in other sea urchins (Crapp and Willis 1975, Bernard 1977, Byrne 1990, King et al. 1994), is likely related to individual differences in the acquisition and allocation of energy reserves to gametogenesis. To my knowledge, my study is the first to quantitatively document changes in cell type abundance in the gonads of *S. droebachiensis*, and thus serves as a benchmark for future histological studies of the reproductive cycle of this species.

A more gradual decline in gonad index during the spring spawning period at Mill Cove compared to Little Duck Island suggests that spawning was more protracted or occurred somewhat later at the former site. In the Northwestern Atlantic, spawning of *Strongylocentrotus droebachiensis* is triggered by phytoplankton blooms (Himmelman 1975, Starr et al. 1990, 1992, 1993) which vary in space and time. Differences in temperature or hydrodynamic regimes between my sites may have influenced the occurrence of phytoplankton blooms and hence the timing of sea urchin spawning.

Histological analysis revealed that a small proportion of the population of *Strongylocentrotus droebachiensis* at Mill Cove spawned in fall. Although the incidence of summer and fall spawning is low, it corroborates observations by Keats et al. (1987) of spawning of *S. droebachiensis* in June, July and September in barrens in Newfoundland. Because of the low number of sea urchins that may spawn in the summer or fall, it is unlikely that these events would contribute much to the overall pool of larvae produced each year.

Spatial and interannual variation in gonad index

Gonad indices of *Strongylocentrotus droebachiensis* generally were higher in the kelp bed and grazing front than in the barrens. This pattern is consistent with previous studies contrasting the gonad index of this species (Lang and Mann 1976, Keats et al. 1984, Scheibling and Stephenson 1984, Sivertsen and Hopkins 1995) or other strongylocentrotids (Gonor 1973a, Pearse 1980) between kelp beds and barrens, and presumably is related to differences in food availability (see 'Between habitat variation in food consumption', below). Several studies have shown that laminarian kelps are a preferred food of *S. droebachiensis* which supports high rates of growth and reproduction (Vadas 1977, Keats et al. 1984, Lemire and Himmelman 1996, Minor and

Scheibling 1997). My histological analysis indicated that the gonads were qualitatively similar between habitats (in terms of the proportions of different cell types) despite large differences in gonadal mass. In contrast, Minor and Scheibling (1997) found that females of *S. droebachiensis* fed kelp (*Laminaria longicruris*) ad libitum in the laboratory had significantly more nutritive phagocytes in their gonads than those fed kelp only one day per week, and suggested that the higher ration provided additional reserves for gametogenesis. However, greater between-diet differences in gonad production in the laboratory study may account for this disparity.

The peak gonad index increased between 1993 and 1995 in the barrens at Little Duck Island, which may reflect a reduction in intraspecific competition for food after the mass mortality in October 1993 (Scheibling and Hennigar 1997). There were no interannual differences in peak gonad index in the kelp bed or grazing front during this period, suggesting that food supply (mainly kelp) was not limiting reproduction in either of these two habitats. Other studies comparing gonad indices over several years also have shown interannual differences in peak gonad index (Himmelman 1978, Keats et al. 1984, Munk 1992) which in some cases were related to differences in food supply (Keats et al. 1984).

Gonad indices usually were higher at the wave-exposed site, Little Duck Island, than at the sheltered site, Mill Cove. In contrast, Ebert (1968) and Gonor (1973a) found that *Strongylocentrotus purpuratus* had lower gonad indices at exposed sites than at sheltered sites, which Ebert attributed to greater energy allocation to spine repair at exposed sites. In both studies, however, differences in wave exposure were confounded with differences in food abundance, which was lower (Ebert 1968) or higher (Gonor 1973a) at the sheltered site.

Between habitat variation in food consumption

At both sites, the quantity of the gut contents of *Strongylocentrotus droebachiensis* was lowest in late summer and early fall when gonad indices also were low. This suggests a decrease in feeding rate at this time which is consistent with observations of sea urchin behaviour at Little Duck Island and Mill Cove during the period of study: sea urchins in the grazing front became less aggregated and grazed less actively on kelp in the late summer and fall (Scheibling et al. submitted). Gut contents at Little Duck Island also were relatively low at the peak of the reproductive cycle but increased after spawning. Previous studies of *S. droebachiensis* (Vadas 1977, Himmelman 1980, Keats et al. 1983, Himmelman and Nédélec 1990) and congeneric species (Lawrence et al. 1965, Ebert 1968, Vadas 1977) also have shown a decline in feeding rate in late summer/early fall with a minimum around the peak of the reproductive cycle. The large differences in the abundance of macroalgal food resources between kelp beds and barrens were not reflected in large differences in the quantity of gut contents of sea urchins from these habitats. However, as sea urchins decrease gut evacuation rate when food is scarce (Lasker and Giese 1954, Propp 1977), the quantity of gut contents in barrens may not adequately reflect the level of food consumption or availability. Therefore, a significant difference may exist in the quantity of food consumed between barrens and kelp beds which I was unable to detect.

At both sites, food quality in terms of organic material tended to be lower in the barrens than in the kelp bed or at the grazing front. Vadas (1977) found that food quality is more important than quantity for reproduction in *Strongylocentrotus droebachiensis*, which may explain the lower gonad index of urchins in barrens. Nevertheless, sea urchins in barrens are able to obtain sufficient nutrients for growth and reproduction owing to their generalist diet and ability to locate and consume drift algae such as kelps

(Himmelman and Steele 1971, Lawrence 1975, Vadas 1977, Mann et al. 1984, Keats et al. 1984). My study described in Chapter 3 showed that the growth rate of adult sea urchins did not differ significantly among habitats at Little Duck Island, although it was somewhat slower in the barrens than in the kelp bed or grazing front at Mill Cove. If sea urchins channel a similar proportion of energy into growth in all habitats, reduced energy intake in barrens should result in reduced reproduction. Also, foraging costs may be higher in barrens where individuals tend to move greater distances than in kelp beds or grazing fronts (Mattison et al. 1977, Harrold and Reed 1985, Scheibling unpubl. data), which would further reduce the amount of energy available for reproduction.

Sex ratio and sexual differences in gonad index

The sex ratio of *Strongylocentrotus droebachiensis* approximated 1:1 in all cases except for the kelp bed at Little Duck Island, where males accounted for a slightly higher percentage of the population (56%). Munk (1992) also reported a slight bias towards males in one population of *S. droebachiensis* in Alaska (59%), but a slight bias towards females in another population (56%). Biased sex ratios have also been reported for congeneric species (Gonor 1973c, Bernard 1977), although gonochoric echinoderms such as strongylocentrotids typically have a sex ratio of 1:1 (Lawrence 1987). The incidence of hermaphroditism in my study was very low and similar to that found in other gonochoric sea urchins (Bernard 1977, Lawrence 1987, Byrne 1990, King et al. 1994).

At the peak of the reproductive cycle in spring 1995, females had a higher gonad index than males at both sites, which is consistent with previous studies of *S. droebachiensis* (Munk 1992, Minor and Scheibling 1997) but not other strongylocentrotids (Bennett and Giese 1955, Bernard 1977). After spawning, gonad

indices of both sexes dropped to the same minimal levels, indicating that females released a larger proportion (~10.5%) of their body weight as gametes than males (~8.1%).

Spatial variation in zygote production

A number of studies have shown that fertilization rate in sea urchins and other echinoderms is positively related to fecundity (which generally increases with increasing body size) and population density (e.g., Pennington 1985, Levitan et al. 1992, Levitan 1995 and references therein). Adults of *Strongylocentrotus droebachiensis* in barrens have low fecundity (because of their small size and low gonad index) while those in kelp beds have a high gonad index but are sparsely distributed. In contrast, sea urchins in grazing fronts are both highly aggregated and much larger than those in barrens and kelp beds (Scheibling et al. 1994, Scheibling et al. submitted) and therefore are expected to have the highest fertilization rate and produce the greatest number of zygotes per unit area of bottom. During my study, sea urchins at Little Duck Island had higher fecundity and occurred at higher densities than those at Mill Cove. Consequently, sea urchins at Little Duck Island probably also experienced higher fertilization success and produced more zygotes per unit area. The hypothesis that the number of zygotes produced per unit area differs among sea urchins in different habitats was further explored in my theoretical work (Chapter 6).

Chapter 3: Size and age structure of sea urchins in different habitats

INTRODUCTION

The shallow rocky subtidal zone along temperate coastlines is usually characterised by the presence of extensive and highly productive kelp beds (Mann 1972, Harrold and Pearse 1987). Off the Atlantic coast of Nova Scotia, however, kelp beds (*Laminaria longicruris* and *L. digitata*) are periodically destroyed during population outbreaks of the dominant herbivore, the sea urchin *Strongylocentrotus droebachiensis*. As sea urchins increase in abundance, they form dense feeding aggregations (fronts) along the borders of kelp beds, which advance as a cohesive unit, creating barren grounds devoid of fleshy macroalgae in their wake (Breen and Mann 1976b, Mann 1977, Miller 1985b, Scheibling 1984, 1986, 1994).

Subpopulations of sea urchins in kelp beds, barrens, and in grazing fronts at the interface of these two habitats have distinctive characteristics. Sea urchin density is very high along the front, intermediate in barrens and low in kelp beds (Lang and Mann 1976, Bernstein et al. 1981, Scheibling et al. submitted). Reproductive output is higher in sea urchins in kelp beds and in grazing fronts than in those inhabiting barrens (Johnson and Mann 1982, Keats et al. 1984, Chapter 2). Sea urchins in fronts are also much larger than those in barrens, while those in kelp beds are of variable size (Lang and Mann 1976, Witman et al. 1982). Since growth rate of sea urchins is largely controlled by the quantity and quality of available food (Ebert 1968, Lawrence 1975, Vadas 1977, Larson et al. 1980, Thompson 1982, 1984, de Jong-Westman et al. 1995a, Minor and Scheibling 1997), these size differences may be related to the large differences in macroalgal abundance between kelp beds and barrens. However, barrens

also receive substantial inputs of drift algae (Johnson and Mann 1982, Keats et al. 1984), which may enable sea urchins in this habitat to achieve similar growth rates as those in kelp beds or grazing fronts.

Differences in size structure among subpopulations of *S. droebachiensis* also may be related to differences in age structure. Differences in age distributions of sea urchins among habitats may be related to higher rates of settlement and recruitment of sea urchins in barrens than in kelp beds (Raymond and Scheibling 1987, Balch and Scheibling 1998, Balch et al. 1998). Also, age-specific differences in aggregation and sheltering behaviours of sea urchins (Vadas 1977, Larson et al. 1980, Bernstein et al. 1981) may result in different age distributions among subpopulations in different habitats.

My study examines the relationship between size and age of sea urchins in subpopulations in kelp beds, grazing fronts, and barrens to determine whether differences in size structure are due to differential growth rates and/or differences in age structure.

MATERIALS AND METHODS

I compared subpopulations of *Strongylocentrotus droebachiensis* in kelp (*Laminaria longicruris*) beds, grazing fronts and barrens at the two study sites described in Chapter 2, Little Duck Island in Mahone Bay and Mill Cove in St. Margaret's Bay. Sea urchins in each subpopulation were sampled in haphazardly placed 0.25 m^2 quadrats using SCUBA in March/April 1995. I carefully inspected cracks and crevices, and the undersides of cobbles and boulders, to ensure that small and cryptic individuals were representatively sampled.

For determination of size structure, I collected sea urchins >5 mm test diameter in 10-15 quadrats within each subpopulation. Horizontal test diameter of all sea urchins in a sample was measured with vernier calipers (0.05 mm accuracy).

Sea urchins were aged using Robinson and MacIntyre's (1997) modification of Jensen's (1969) technique. Rotules of 59-71 sea urchins from each subpopulation were dissected from the Aristotle's lantern, charred over an alcohol flame, embedded in a mounting agent (Crystalbond™ 509, Aremco Products, Inc., Ossining, New York, U.S.A.) and sanded with fine grit paper to the central longitudinal plane (Fig. 3.1). Examination under a dissecting microscope revealed wide, light rings produced during periods of fast growth in the summer, and narrow, dark rings produced during periods of slow growth in the winter. Thus, paired light and dark rings indicate one year's growth. Robinson and MacIntyre (1997) validated the annual nature of the rings by marking sea urchins with oxytetracyline and recording growth rings 14 months later. They also calibrated the rate of ring formation against the calcium: magnesium ratio in the rotules, which shows a distinct seasonal pattern.

Logistic growth curves were derived from size-at-age data using the function:

$$D = \frac{D_0 N}{D_0 + (N - D_0) e^{-gNY}} \qquad [3.1]$$

where D is test diameter, D_0 is test diameter at settlement, N is asymptotic test diameter, g is a constant, and Y is age. Statistical comparisons of growth curves were based on linear regressions, which provided a good fit to my data. Slopes of growth (size-at-age) curves and Y-intercepts were compared using ANCOVA with age as the covariate. Post hoc comparisons were made using the Student-Newman-Keuls (SNK) test (α=0.05). Size distributions in each subpopulation were translated into age distributions using

linear regressions (Table 3.1). I compared size and age distributions of *S. droebachiensis* between subpopulations and sites using Kolmogorov-Smirnov tests.

To compare the diet of *S. droebachiensis* in kelp beds, barrens and grazing fronts, I analysed the gut content of sea urchins collected in 10 quadrats in each subpopulation. Gut content analysis of adults (>20 mm test diameter) was based on 11-13 sample dates between April 1994 and August 1995 with 6-74 sea urchins analysed at each date. Gut content analysis of juvenile sea urchins (<20 mm) was based on a single sample in April 1995 at Little Duck Island and in March 1995 at Mill Cove. Food quantity was expressed as the ratio of food volume in guts to total body volume. Food quality was measured by estimating the percentage of the total gut content that was organic matter (remains of fleshy macroalgae). Comparisons of subpopulations were done by one-way ANOVA.

RESULTS

Size structure of subpopulations of *Strongylocentrotus droebachiensis* differed between habitats and sites (Fig. 3.2). At Little Duck Island, the size distributions of sea urchins were approximately normal in all habitats with the largest sea urchins in the grazing front and the smallest in the barrens. At Mill Cove, sea urchins were normally distributed and largest in the front. Size distributions in the kelp bed and barrens were skewed towards larger sizes, with larger sea urchins in the kelp bed. Within each subpopulation, sea urchins were larger at Little Duck Island than at Mill Cove. Differences between subpopulations within sites and within subpopulations between sites were all statistically significant (Kolmogorov-Smirnov tests, $p<0.001$).

Size-at-age data and logistic growth curves are shown in Fig. 3.3. Sea urchins in this study were 2-9 years old, with the majority (94%) being 3-7 years old, although

variation in test diameter at a given age is considerable at both sites and in each subpopulation. Logistic growth curves based on size-at-age data (see Table 3.2 for equation parameters) included mainly adult sea urchins (>2 years old). Therefore, the test diameter at settlement (D_0=2) based on the best fit to the data clearly overestimates the actual size at settlement (~0.3 mm; Raymond and Scheibling 1987, Chapter 5). In kelp beds and grazing fronts at both sites, the growth curves show that sea urchins ≤2 years old grow slowly, sea urchins 3-5 years old grow rapidly, and those ≥6 years old have reached asymptotic size. In barrens, sea urchins ≤3 and ≥7 years old grow more slowly than those 4-6 years old. Sea urchins in the barrens appear to reach asymptotic size at a later age than those in the kelp bed and the grazing front.

Slopes and Y-intercepts of growth curves were compared based on linear regressions of test diameter on age (Table 3.3). At Little Duck Island, the slopes of linear regressions, which mainly reflect growth rates of adults, did not differ significantly between habitats ($F_{2,183}$=0.54, p=0.584). However, the Y-intercepts of growth curves, which indicate differences in growth rates of juveniles, differed significantly between habitats ($F_{2,185}$=41.1, p<0.001): the intercept in the barrens was significantly lower than that in the kelp bed and front (SNK, p<0.05) which were similar to each other (p>0.05). At Mill Cove, the slopes of linear regressions were at the critical significance level ($F_{2,198}$=3.05, p=0.050) suggesting that the slopes differed between habitats. Pairwise comparisons showed that the slope in the barrens was significantly lower than that in the kelp bed ($F_{1,129}$=5.57, p=0.020). The slope in the barrens also was lower than that in the front ($F_{1,132}$=3.46, p=0.065), although this difference was marginally non significant. The regression slopes did not differ significantly between the kelp bed and grazing front ($F_{1,135}$=0.95, p=0.332).

Differences in age structure of subpopulations of *S. droebachiensis* (Fig. 3.4) reflected differences in size structure (Fig. 3.2). At Little Duck Island, age structure was similar in the kelp bed and barrens (Kolmogorov-Smirnov test, $D_{194,218}$=0.105, p=0.207), and in both habitats sea urchins were younger than in the grazing front (p<0.001). At Mill Cove, differences between subpopulations were all significant (p<0.001). Also, differences within subpopulations between sites were significant (p<0.001).

Gut fullness of adult sea urchins when pooled over sampling dates (Fig. 3.5a, b) did not differ significantly among subpopulations at each site (Little Duck Island: $F_{2,30}$=2.38, p=0.11; Mill Cove: $F_{2,33}$=1.01, p=0.374; also see Chapter 2). However, organic gut content of adults was significantly (SNK, p<0.05) lower in the subpopulations in the barrens than in those in the kelp bed and grazing front (Little Duck Island: $F_{2,36}$=7.50, p=0.002; Mill Cove: $F_{2,36}$=19.66, p<0.001; also see Chapter 2). Organic gut content of juveniles (Fig. 3.5c) also differed significantly between subpopulations at each site (Little Duck Island: $F_{2,15}$=6.16, p=0.011; Mill Cove: $F_{2,16}$=12.29, p=0.001). At Little Duck Island, organic gut content of sea urchins did not differ significantly between subpopulations in the grazing front and barrens (SNK, p>0.05), both of which had a significantly lower organic gut content than the subpopulation in the kelp bed (p<0.05). At Mill Cove, organic gut content was significantly lower in juveniles in the barrens than in those in the kelp bed and the grazing front (p<0.05), which did not differ significantly.

Table 3.1. Linear regressions ($Y=bD$) of age (Y, years) on test diameter (D, mm). [b, slope; n, sample size; r^2, coefficient of determination]

Site/Habitat	b	n	r^2
Little Duck Island			
Kelp bed	0.111	194	0.622
Grazing front	0.122	269	0.802
Barrens	0.150	218	0.773
Mill Cove			
Kelp bed	0.124	348	0.426
Grazing front	0.133	198	0.706
Barrens	0.161	336	0.406

Table 3.2. Parameter values of equation 3.1 (see Materials and Methods) used to generate logistic growth curves (Fig. 3.3). [D_0, test diameter at settlement; N, asymptotic test diameter; g, a constant; r^2, coefficient of determination]

Site/Habitat	D_0	N	g	r^2
Little Duck Island				
Kelp bed	2	52	0.020	0.611
Grazing front	2	56	0.018	0.787
Barrens	2	54	0.014	0.738
Mill Cove				
Kelp bed	2	47	0.021	0.624
Grazing front	2	52	0.017	0.722
Barrens	2	45	0.017	0.470

Table 3.3. Linear regressions ($D=a+bY$) of test diameter (D, mm) on age (Y, years). [a, intersept; b, slope; n, sample size; r^2, coefficient of determination]

Site/Habitat	a	b	n	r^2
Little Duck Island				
Kelp bed	7.94	6.99	65	0.649
Grazing front	8.49	6.49	58	0.802
Barrens	2.00	6.22	66	0.798
Mill Cove				
Kelp bed	1.80	7.42	68	0.580
Grazing front	4.67	6.55	71	0.736
Barrens	5.49	5.14	65	0.549

Fig. 3.1. Prepared rotule of 8-year old sea urchin with dark (8) and light (7) age rings. Scale bar is 1 mm.

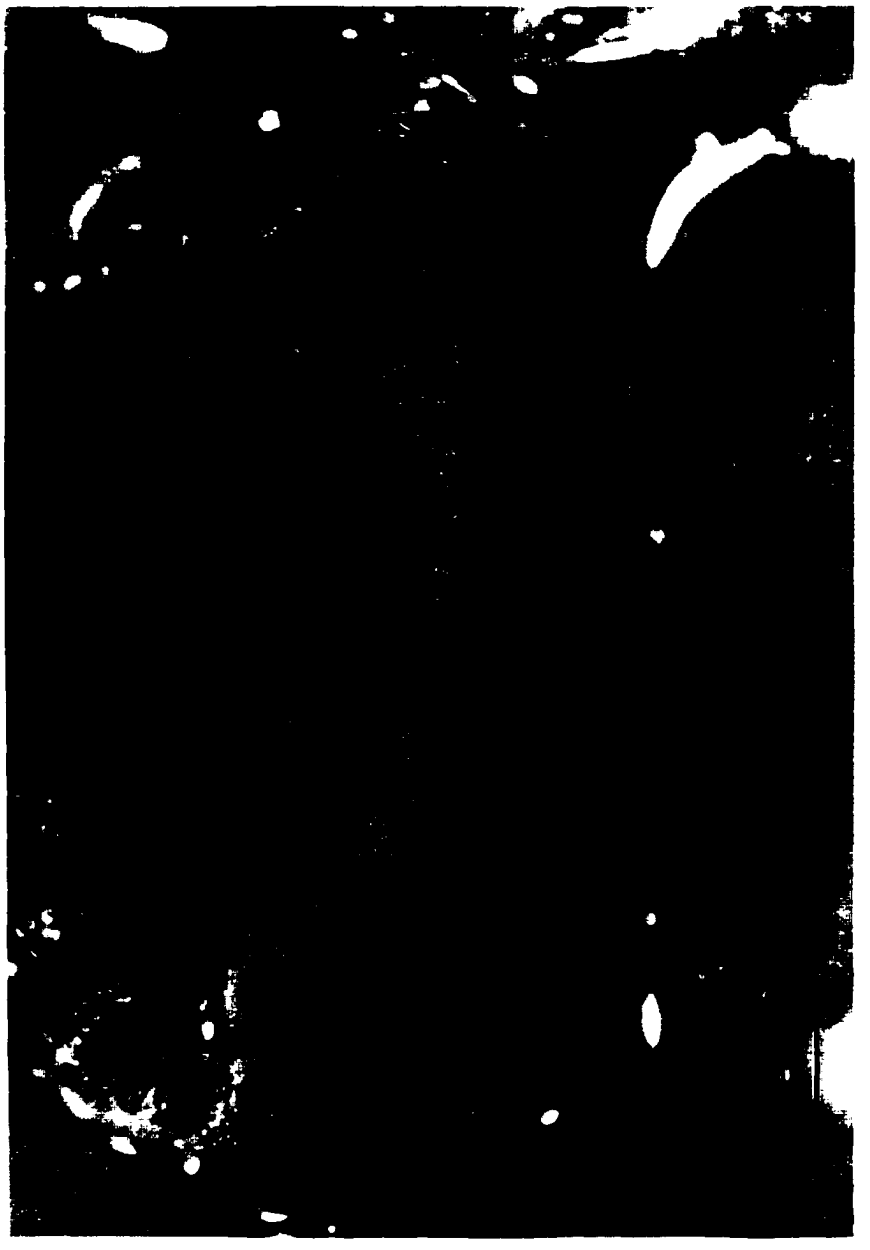

Figure 3.1

Fig. 3.2. Size structure of subpopulations in kelp beds, grazing fronts and barrens at Little Duck Island and Mill Cove in March/April 1995. [n, sample size; med., median test diameter (mm).]

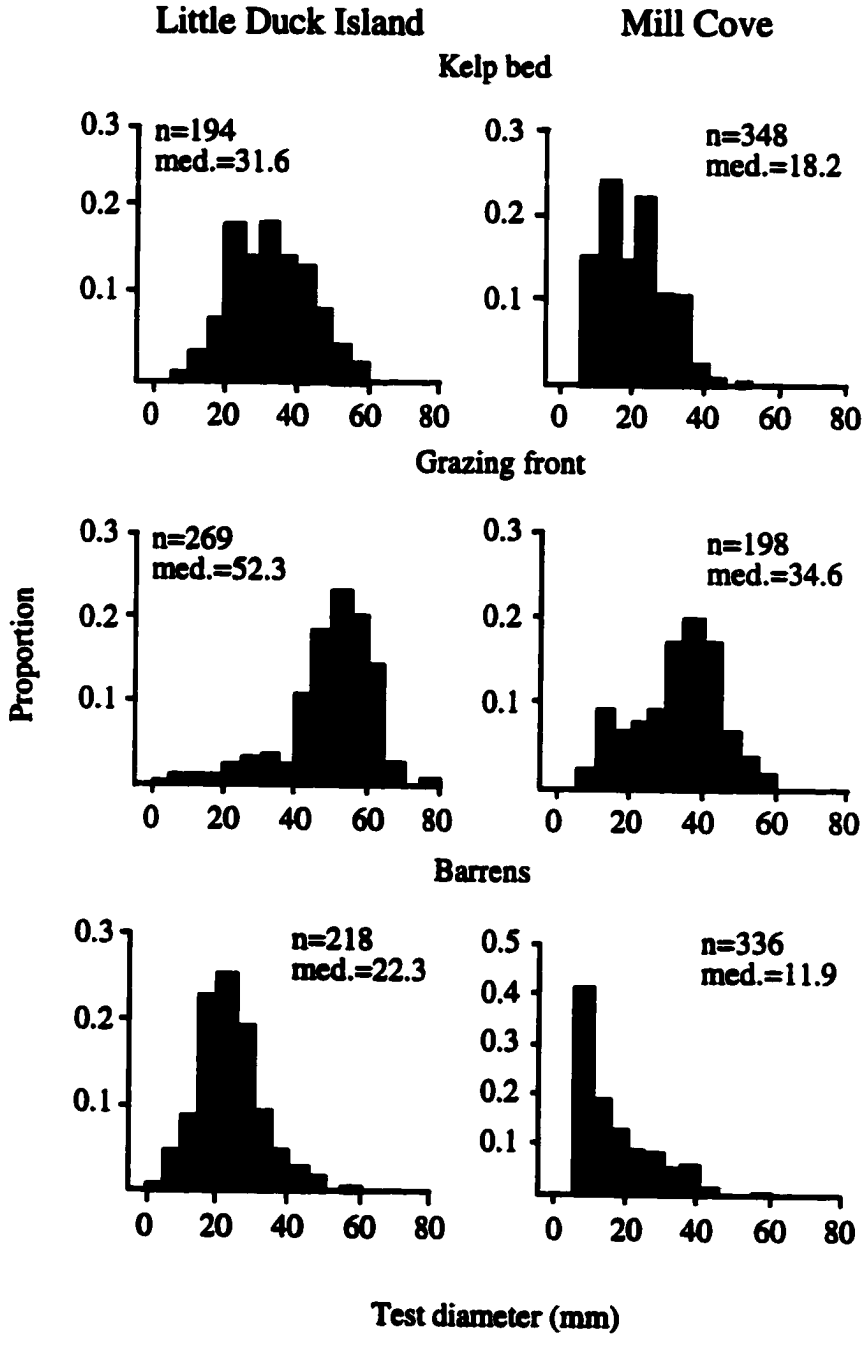
Little Duck Island
Mill Cove
Kelp bed
Grazing front
Barrens
n=194
med.=31.6
n=348
med.=18.2
n=269
med.=52.3
n=198
med.=34.6
n=218
med.=22.3
n=336
med.=11.9
Proportion
Test diameter (mm)

Figure 3.2

Fig. 3.3. Size-at-age data from March/April 1995 in kelp beds, grazing fronts and barrens at Little Duck Island and Mill Cove. Data are fitted with logistic growth curves (generated using equation 3.1, see Table 3.2 for parameter values). (n, sample size; r^2, coefficient of determination.)

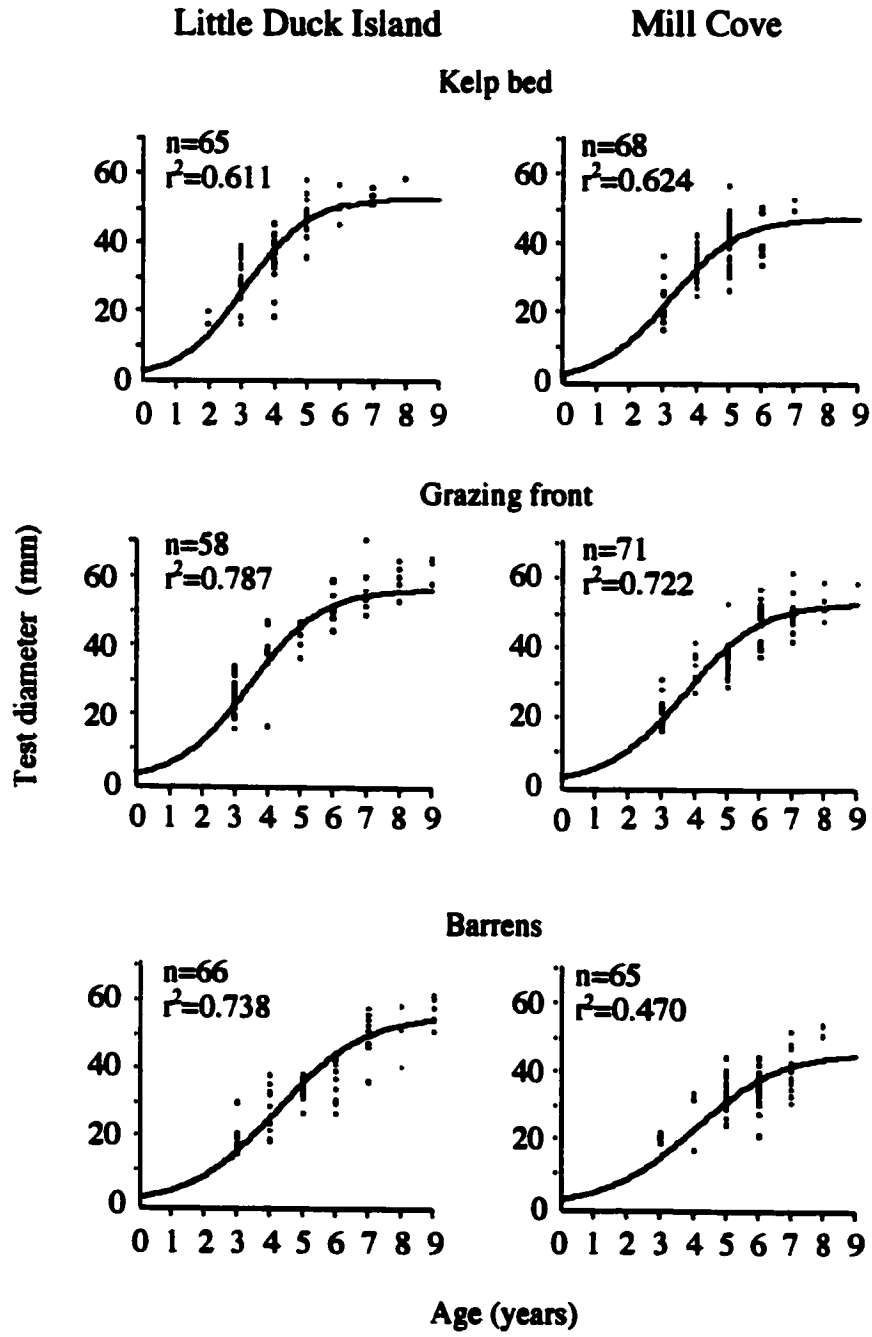
Little Duck Island
Mill Cove
Kelp bed
Grazing front
Barrens
n=65
r²=0.611
n=68
r²=0.624
n=58
r²=0.787
n=71
r²=0.722
n=66
r²=0.738
n=65
r²=0.470
Test diameter (mm)
Age (years)
0
20
40
60
0 1 2 3 4 5 6 7 8 9

Figure 3.3

Fig 3.4. Age structure in March/April 1995 in kelp beds, grazing fronts and barrens at Little Duck Island and Mill Cove. Age structure was derived from size structure (Fig. 3.2) using linear regression (Table 3.1). [n, sample size; med., median age (years).]

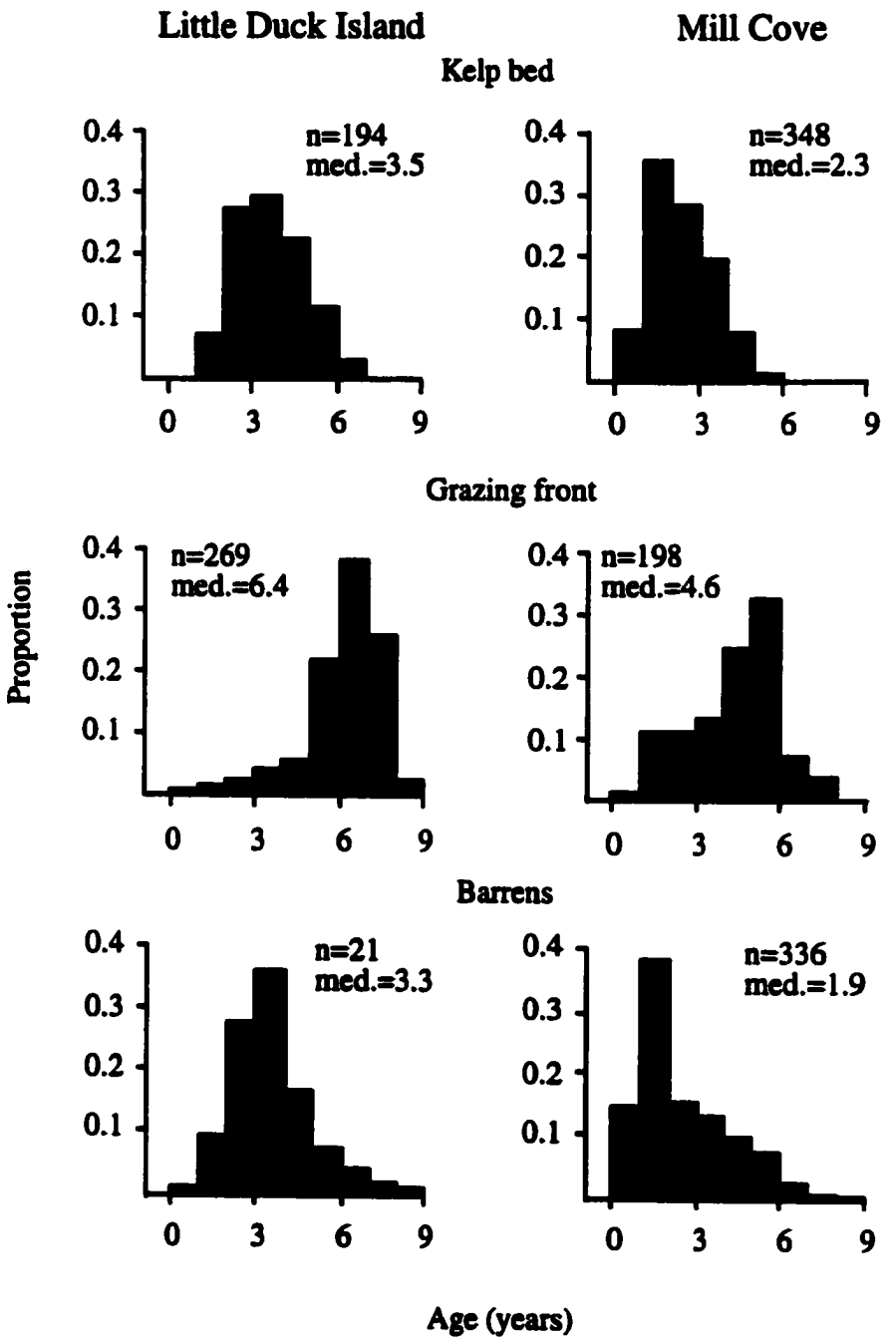
Little Duck Island
Mill Cove
Kelp bed
n=194
med.=3.5
n=348
med.=2.3
Grazing front
n=269
med.=6.4
n=198
med.=4.6
Barrens
n=21
med.=3.3
n=336
med.=1.9
Proportion
0.4
0.3
0.2
0.1
0
3
6
9
Age (years)

Figure 3.4

Fig. 3.5. a) Gut fullness (% of body volume) of adults, b) Organic gut content (% of total gut content) of adults, and c) Organic gut content of juveniles in kelp beds, grazing fronts and barrens at Little Duck Island and Mill Cove. Data for adults are grand means (+SD) of means from 11-13 sampling dates. Data for juveniles are means (+SD) of samples collected in March/April 1995. Numbers above bars indicate number of sampling dates (a, b) or sample size (c). (LDI, Little Duck Island; MC, Mill Cove.)

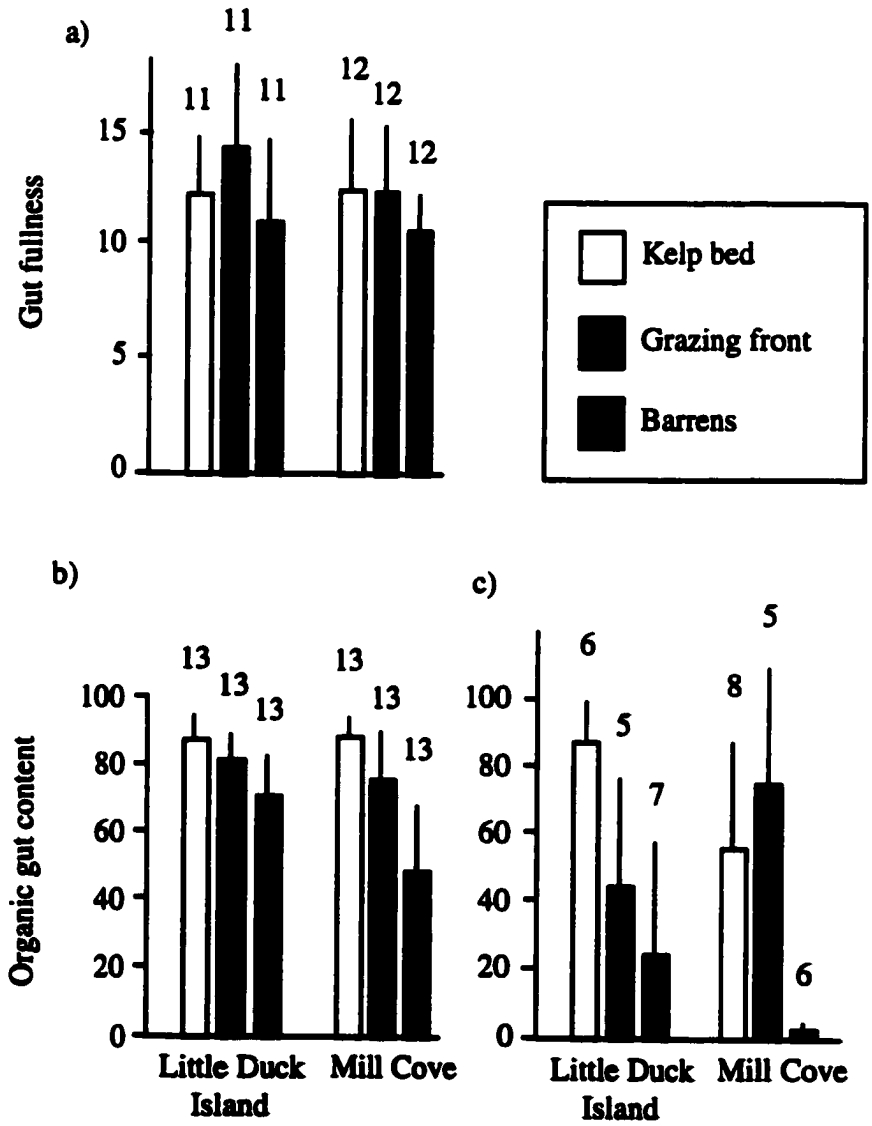
a)
Gut fullness
0
5
10
15
11
11
11
12
12
12
Kelp bed
Grazing front
Barrens
b)
Organic gut content
0
20
40
60
80
100
13
13
13
13
13
13
Little Duck Island
Mill Cove
c)
0
20
40
60
80
100
6
5
7
8
5
6
Little Duck Island
Mill Cove

Figure 3.5

DISCUSSION

Analysis of my size-at-age data (Fig. 3.3) showed that the overall growth rate of *Strongylocentrotus droebachiensis* was faster in kelp beds and in grazing fronts along the border of kelp beds than in barrens. This difference is mainly attributable to differences in the growth rates of juveniles, as growth of adults was similar among subpopulations, with the exception of the barren gounds at Mill Cove where it was lowest. This finding is consistent with previous studies of growth in adults of *S. droebachiensis* (Lang and Mann 1976, Wharton and Mann 1981, Himmelman et al. 1983a, Raymond and Scheibling 1987, Leinaas and Christie 1996).

Since growth in sea urchins is largely determined by food quality and quantity (Vadas 1977), can differences in diet explain the observed differences in overall growth rates? Gut fullness of *S. droebachiensis* was similar among subpopulations while organic matter in gut contents was typically lower in barrens than in kelp beds and grazing fronts, particularly for juvenile sea urchins. Juveniles are usually cryptic, feeding on coralline algae, microalgae, and macroalgal detritus under rocks and in crevices (Keats et al. 1985, Raymond and Scheibling 1987). In barrens, the diet of juveniles consists mostly of coralline algae (Hagen 1983, Himmelman 1986, Himmelman and Nédélec 1990, Guillou and Michel 1993), which have a lower nutritional value than fleshy macroalgae such as kelps (Larson et al. 1980), suggesting that growth of juvenile sea urchins in barrens is food limited (Himmelman et al. 1983a, Himmelman 1986, Raymond and Scheibling 1987). Adult sea urchins are highly mobile and have the ability to detect and locate drift algae (Lawrence 1975, Vadas 1977, Mann et al. 1984, Himmelman and Nédélec 1990), which may account for a substantial proportion of the diet of sea urchins in barrens (Johnson and Mann 1982, Keats et al. 1984). The relatively large amounts of organic matter I found in guts of adult sea

urchins in the barrens at both sites indicate a good supply of drift algae to this habitat. At Little Duck Island, energy derived from drift algae seemed sufficient to allow adult sea urchins in barrens to grow at a similar rate as those in kelp beds and in grazing fronts. At Mill Cove, however, reduced energy intake resulted in a reduced growth rate of adult sea urchins in the barrens relative to those in the kelp bed and grazing front.

In adult sea urchins, energy is allocated to both growth and reproduction (Thompson 1979), and differences in food quantity and quality between kelp beds and barrens appear to have a greater effect on reproductive output than on growth rate, as gonad indices always are lower in barrens than in kelp beds and grazing fronts (Lang and Mann 1976, Hagen 1983, Keats et al. 1984, Raymond and Scheibling 1987, Chapter 2). Also, reduced energy intake in barrens may be compounded by increased foraging costs (Mattison et al. 1977), further reducing the amount of energy available for reproduction and growth. My finding that sea urchins in barrens can maintain a high growth rate at the expense of gonadal output appears to contradict Thompson's (1982) finding that *S. droebachiensis* on a low feeding ration maintain high gonadal output at the expense of growth. However, Thompson's study was conducted in the laboratory with sea urchins fed a kelp diet enhanced with mussel flesh, which limits comparability to my study.

The observed differences in overall growth rates between subpopulations do not explain the observed differences in size structure. When size structure is translated into age structure, it is clear that sea urchins in grazing fronts are older than those in kelp beds and barrens. Differences in age structure between kelp beds and barrens are consistent with previous reports for *S. droebachiensis* in Nova Scotia (Lang and Mann 1976) and Norway (Leinaas and Christie 1996). The age structure of sea urchins in kelp beds and barrens indicates that recruitment occurs in both habitats, but is higher in

barrens. Very few juvenile sea urchins occur in the grazing front. Numerous studies have shown that adult *S. droebachiensis* aggregate in response to food and predators (Vadas 1977, Bernstein et al. 1981, Miller 1985b) and that this behaviour can lead to the formation of grazing fronts. The older age of sea urchins in a front suggests that a front is a cohesive unit that originally forms in deeper water and migrates into the shallows (Scheibling et al. submitted). The low number of young/small sea urchins in a front may also be due to their exclusion from the front by larger animals (Lang and Mann 1976) and/or to differential emigration/immigration in a front, which can further reinforce a given age/size structure.

Chapter 4: Effects of food type and ration on reproductive maturation and growth of sea urchins

INTRODUCTION

In many species of marine invertebrates, age (or size) at first reproduction varies among habitats and populations. Gonad production, in relative terms, is also variable but often increases exponentially in young adults. Both maturation rate and gonad production have a profound effect on the reproductive value of an individual and, by extension, of a population. For sea urchins, field studies indicate that animals in habitats with abundant food (e.g., kelp beds) may become reproductively mature at an earlier age and (or) a smaller size than sea urchins in habitats with little food (e.g., barrens) (Buchanan 1966, Sivertsen and Hopkins 1995). Growth rate, which determines absolute gonad size, also affects reproduction. Numerous field and laboratory studies have demonstrated a positive effect of food quality and quantity on growth rate of juveniles (Swan 1961, Raymond and Scheibling 1987, Chapter 3), and reproductive output and growth rate of adults (Ebert 1968, Dix 1972, Vadas 1977, Larson et al. 1980, Andrew 1986, Byrne 1990, Lawrence et al. 1994, Chapters 2, 3).

Although sea urchins exhibit strong food preferences (Lawrence 1975, Vadas 1977, Vance and Schmitt 1979, Larson et al. 1980, DeRidder and Lawrence 1982), they are generalist feeders when preferred foods are scarce. For example, *Strongylocentrotus droebachiensis* feeds preferentially on laminarian kelps, but often includes in its diet other algae, such as encrusting corallines (Himmelman and Steele 1971, Foreman 1977, Chapter 2), or material of animal origin (Himmelman and Steele 1971, Chapman 1981, Witman 1985, Sebens 1985). Laminarian kelps yield high rates of growth and reproduction when used as a sole source of food (e.g., Vadas 1977, Keats et al. 1983,

1984, Himmelman 1984, Minor and Scheibling 1997); however, field observations and laboratory experiments have indicated that an addition of animal matter (a source of protein) to the diet can further enhance somatic and gonadal growth (Levin and Naidenko 1987, Lawrence et al. 1992, Fernandez and Caltagirone 1994, Nestler and Harris 1994, de Jong-Westman et al. 1995a). In contrast, encrusting coralline algae, which have a low energy content (Paine and Vadas 1969), support little or no growth or reproduction (Keats et al. 1983, 1984, Lemire and Himmelman 1996).

Strongylocentrotus droebachiensis is the dominant herbivore in the shallow rocky subtidal zone in Eastern Canada (Miller and Mann 1973, Mann 1977). Major fluctuations in the abundance of this species have caused large-scale transitions in the subtidal community state from kelp beds to sea urchin-dominated barrens (Chapter 1). During a population outbreak, sea urchins form dense aggregations at the edges of kelp beds and destructively graze macroalgae, thereby creating barrens devoid of fleshy macroalgae (Breen and Mann 1976b, Scheibling et al. 1994). Variation in reproductive output of *S. droebachiensis* among different community states, and hence variation in larval production, may play an important role in the population dynamics of this species in Eastern Canada (Minor and Scheibling 1997).

In this study, I examine the effects of food type and ration on reproductive maturation, gonadal and somatic growth, and survival of juveniles and young adults of *Strongylocentrotus droebachiensis* in a laboratory experiment. Understanding specific dietary effects on these variables can provide insight into the consequences of habitat transitions on the reproductive ecology of *S. droebachiensis* and the dynamics of population outbreaks. Furthermore, considering the increasing interest in sea urchin aquaculture in Eastern Canada (Hatcher and Hatcher 1997), the effects of diet on growth

and maturation of juvenile sea urchins could be used to develop effective aquacultural practices for this important resource.

MATERIALS AND METHODS

Experimental design

To investigate the effects of food quality and quantity on reproductive maturation and growth of juveniles of *Strongylocentrotus droebachiensis*, I conducted a 22-month (19 May 1995 to 21 March 1997) feeding experiment in laboratory aquaria. Juvenile sea urchins 13-17 mm in horizontal test diameter (2-3 years old; Chapter 3) were collected from a sea urchin-dominated barren ground off Little Duck Island (see Chapter 2 for a description of the collection site) on 17 May 1995, shortly after the annual spawning period (March/April; Cocanour and Allen 1967, Himmelman 1978, Chapter 2). *Strongylocentrotus droebachiensis* of this size can grow 7-17 mm per year (Raymond and Scheibling 1987, Munk 1992, Chapter 3) and become reproductively mature at 18-25 mm (Vadas 1977, Thompson 1979, Raymond and Scheibling 1987, Munk 1992). Therefore, the experimental animals had the potential to mature in the following reproductive season (hereafter termed 'first reproduction'). Prior to the experiment, the sea urchins were held without food in flowing sea water tanks for ~36 h after collection. Rocks encrusted with coralline red algae (*Phymatolithon laevigatum* and *Lithothamnion glaciale*, collected at the same time as the sea urchins) were supplied once at the start of the experiment as a standardized food source. Experimental diets, in order of decreasing food quality and/or quantity, were: 1) kelp (blades of *Laminaria longicruris* and *L. digitata*) for 6 d wk^{-1} and mussels (flesh of *Mytilus edulis* and *M. trossulus*) for 1 d wk^{-1} (KM); 2) kelp for 7 d wk^{-1} (high ration, KH); 3) kelp for 1 d wk^{-1} (low ration, KL); and 4) no additional food, aside from coralline algae (NF). In kelp-fed treatments, cut

sections of blades were supplied ad libitum to aquaria for the prescribed feeding periods. In the KM treatment, pieces of mussel flesh were presented to sea urchins individually to evenly distribute this food. Whole kelp plants and live mussels were collected by divers from nearby areas and held in flowing sea water tanks until use. Food rations were increased during the experiment as size and consumption rate of sea urchins increased.

At the start of the experiment, a random sample of 21-22 juvenile sea urchins was added to each of 24 aquaria containing 47 l of flowing sea water (~0.625 l min^{-1}). Aquaria were arranged in 3 tiers with 8 aquaria in each tier. Two replicates of the four feeding treatments were randomly assigned to aquaria within each tier. In April 1996, following removal of sea urchins for analysis at first reproduction or for use in another experiment, the remaining animals were consolidated into 8 aquaria, with 2 replicates of each feeding treatment (11-16 sea urchins in each replicate aquarium) arranged in a single (the middle) tier. The number of aquaria was reduced to maintain similar animal densities and remove the potential for tier effects. Throughout the experiment, sea urchins experienced a natural photoperiod from windows adjacent to the aquaria. Water temperature was measured daily and ranged from 2.3-17.8°C. On any given day, temperature differences among aquaria did not exceed 2.5°C and were usually <1.5°C. Faeces were removed from aquaria 1-2 times per week using a suction hose. All aquaria were scrubbed and rinsed with fresh water four times during the course of the experiment.

Reproduction

At the start of the experiment, a random sample of 20 sea urchins was dissected to confirm their pre-reproductive state. At first reproduction in March 1996, 10 sea

urchins (6 in one case) were randomly selected from each aquarium and dissected to determine gonad index. All remaining sea urchins (14-24 per treatment) were analyzed at second reproduction in March 1997. Wet weights of gonads and the total body were measured with an electronic balance (0.01 g accuracy). Gonad index was calculated as (gonad wet weight / total body wet weight) x 100 to give a percentage. Sex was determined from a gonad smear using a compound microscope.

For histological analysis, I prepared gonads from 2 females and 2 males from each aquarium using standard histological techniques. Serial cross sections (7 μm) cut through the centre of a gonad were stained with haematoxylin and eosin and analyzed using light microscopy and a computerized image analysis system (NIH *Image*, Version 1.60; National Institutes of Health, Bethesda, Maryland, USA). I only analyzed gonadal acini that fit within the frame size of the image analysis system (719 μm^2). For ovaries, I measured the relative abundance (expressed as a percentage of total acinal area) of nutritive phagocytes in 6 randomly selected acini, and the absolute areas of oocytes and ova in a random subsample of 4 acini. Only oocytes sectioned through the nucleolus and ova sectioned through the nucleus were measured. For testes, I measured the relative abundance of nutritive phagocytes, spermatocytes, and spermatozoa in 6 randomly selected acini.

Growth and survival

Horizontal test diameter of all sea urchins was measured with vernier calipers (0.05 mm accuracy) at the start of the experiment and at approximately quarterly intervals throughout the two reproductive cycles. Survival to first and second reproduction was calculated as a percentage based on the number of sea urchins present at the start of each reproductive cycle (i.e., May 1995 and April 1996).

Feeding rate

The feeding rate of sea urchins in the KM, KH and KL treatments was measured in October 1995, and immediately prior to first and second reproduction. A known weight of fresh kelp (0.01 g accuracy) was added to each aquarium and the remainder was removed and re-weighed after 24 h. Because sea urchin size varied greatly among treatments, feeding rate was expressed as the weight of kelp consumed per weight of sea urchins per aquarium. Total wet weight of sea urchins in an aquarium in October 1995 and March 1996 was calculated using a regression (r^2=0.994) of total body wet weight (W, g) on test diameter (D, mm) based on a sample (n=180) from the KM, KH and KL treatments measured in March 1996:

$$\ln W = 2.86 \ln D - 7.16 \qquad [4.1]$$

In March 1997, all sea urchins were weighed and total wet weight in an aquarium was determined directly. To compare feeding rates among treatments on all three dates, I measured the rate in the KM treatment the day before mussel flesh was provided (i.e., on the sixth day of feeding on kelp). Feeding rates in the KH and KL treatments were measured on the day when kelp was provided to the KL treatment. To compare feeding rates among days within the KM treatment, I measured the rate over six consecutive days when kelp was provided. This was done only once in October 1995.

Statistical analysis

Differences in gonad index at first reproduction were compared using a four-way nested analysis of variance (ANOVA) with Treatment (KM, KH, KL) and Sex (female or male; 1-8 replicate sea urchins per sex per aquarium) as fixed factors, and Tier (3 levels) and Aquarium (2 replicate tanks) as random factors. Aquarium was nested within each combination of Treatment and Tier. Tier was designated a random factor as there

was no reason to expect any systematic differences among tiers. Minor and Scheibling (1997), using the same experimental setup, detected no effect of Tier in a similar experiment. At second reproduction, differences in gonad index were compared using a three-way nested ANOVA with the factors Treatment, Aquarium (nested within Treatment), and Sex (3-7 replicate sea urchins per sex per aquarium). The NF treatment was excluded from the first analysis because none of the sea urchins had developed gonadal tissue, and from the second analysis because only three individuals (all female and from one aquarium) could be sexed. We omitted the interaction term Sex x Aquarium from both analyses, and the terms Sex x Tier and Sex x Treatment x Tier from the first analysis, because Minor and Scheibling (1997) showed that these interactions were not statistically significant (at α=0.15) in a similar experimental design. This simplified the analysis and increased power for testing the remaining terms. Unless otherwise noted, the same factor designation, factor levels, and nesting terms were applied in all following analyses. Differences in the gonad index of sea urchins between first and second reproduction were analyzed using three-way ANOVA with the fixed factors Period (first or second reproduction), Treatment (KM, KH, KL), and Sex.

Histological data on the relative abundance of female and male nutritive phagocytes, and male spermatocytes and spermatozoa, were analyzed at first reproduction using a four-way nested ANOVA with Treatment (KM, KH, KL), Tier, Aquarium, and the random factor Individual (2 individuals with 6 replicate measurements per individual; nested within each combination of Treatment, Tier and Aquarium). At second reproduction, the same histological data were compared using a similar three-way nested ANOVA with Treatment (KM, KH, KL; except for analysis of female nutritive phagocytes which included NF as well), Aquarium, and Individual (nested within each combination of Treatment and Aquarium; replication as above).

Absolute areas of oocytes and ova at first reproduction were compared by three-way ANOVA with the factors Treatment (KM, KH, KL), Tier, and the random factor Individual nested within each combination of Treatment and Tier (2-4 individuals with 2-4 replicate measurements per individual for both oocytes and ova). At second reproduction, absolute areas of oocytes and ova were compared using two-way ANOVA with the factors Treatment (KM, KH, KL, NF for oocytes; KM, KH, KL for ova) and Individual (nested within Treatment). There were 4 individuals in KM, KH, and KL, and 2 in NF with 2-4 replicate measurements per individual for oocytes, and 1-4 individuals per treatment and 1-4 replicate measurements per individual for ova. For each individual, replicate measurements represent the average oocyte or ovum area in 1-4 acini. Not all females had measurable oocytes or ova (i.e., those sectioned through the nucleolus or nucleus) resulting in low replication. Therefore I was unable to test for the factor Aquarium and omitted it from the analyses.

Differences in test diameter at the start of the experiment (21-22 replicate sea urchins per aquarium) and at first reproduction [17-22 replicate sea urchins (6 in one case) per aquarium] were compared using three-way nested ANOVA with the factors Treatment (KM, KH, KL, NF), Tier, and Aquarium. At second reproduction, differences in test diameter were compared using a similar two-way nested ANOVA without Tier as a factor and with 7-14 replicate sea urchins per aquarium.

I compared differences in percentage survival among all treatments using one-way ANOVA with 6 replicate aquaria per treatment at first reproduction, and 2 replicate aquaria at second reproduction. One-way ANOVA was also used to compare differences in feeding rates among the KM, KH, and KL treatments, and among days in the KM treatment, with 6 replicate aquaria per treatment in October 1995 and at first reproduction, and 2 replicate aquaria at second reproduction.

Arcsine transformations were applied where necessary (gonad index, percentage survival) to remove heterogeneity of variance as indicated by Cochran's *C* test. Because of some mortality and unequal sex ratios within aquaria, most analyses were unbalanced in terms of replicates. I therefore applied ANOVA procedures for unbalanced data using Type III sums of squares. Because the sums of squares in an unbalanced model are not necessarily independent, the denominator mean square of the *F*-ratio generally is constructed from a linear combination of mean squares, based on the variance components. The denominator degrees of freedom are estimated using the Satterthwaite approximation. More information on these techniques is given in Minor and Scheibling (1997) and references contained therein. Post-hoc comparisons among means (p=0.05) were done using either the Tukey-Kramer test where sample sizes were similar, or the GT2-method (Sokal and Rohlf 1995) where sample sizes were very unequal. For analyses with equal sample sizes (feeding rate and survival), I applied regular ANOVA techniques and used the Student-Newman-Keuls (SNK) test for post-hoc comparisons of means.

RESULTS

Reproduction

A random sample of *Strongylocentrotus droebachiensis* analyzed at the start of the experiment (May 1995) had negligible gonad indices (mean ±SE): female, 1.3 ±0.4% (n=9); male, 0.6% (n=1), unsexed, 0.1 ±0.11% (n=10) (Fig. 4.1). The absence of large oocytes or relict ova in ovaries, and the small size of gonads in all individuals indicated that these sea urchins had not reproduced in the previous spawning season (March/April 1995).

At first and second reproduction, there was a significant effect of Treatment on gonad index (Fig. 4.1; Table 4.1): the index was highest for sea urchins fed kelp and mussels (KM treatment), intermediate for those fed a high ration of kelp (KH treatment), and lowest for those fed a low ration of kelp (KL treatment) (GT2 test, $p<0.05$). At first reproduction, all individuals in the KM and the KH treatment had large gonads (except 1 in KH) and probably would have reproduced in that season. In the KL treatment, 50 out of 60 sea urchins (83%) also appeared to be reproductive; the rest either had small gonads with a few immature sex cells (n=6) or could not be sexed (n=4). At second reproduction, all individuals in kelp-fed treatments were reproductive. Sea urchins which received no food other than a one-time supply of coralline algae (NF treatment) did not develop gonadal tissue at first reproduction, although 10 out of 17 (59%) of these animals had small, immature gonads at second reproduction (the remaining 7 could not be sexed). There was no difference in gonad index between females and males at first reproduction, but females had a significantly higher gonad index at second reproduction: mean gonad index (±SE) pooled over Treatment and Aquarium: females, 28.2 ±2.01%; males, 21.0 ±1.73%. There was no significant interaction of Sex and Treatment at either first or second reproduction. The effect of Aquarium was significant at first but not at second reproduction.

A comparison of gonad indices between first and second reproduction showed a highly significant effect of Period, Treatment, and Sex (Table 4.2). There was also a significant interaction between Period and Sex because gonad index did not differ between sexes at first reproduction, but was higher in females at second reproduction. None of the other interaction terms were significant. Gonad index in both sexes was significantly lower at first than at second reproduction (mean gonad index ±SE pooled

over Treatment: females, 12.9 ±0.02% and 27.3 ±0.07%, respectively; males, 13.0 ±0.02% and 20.1 ±0.06%, respectively; GT2-test, $p<0.05$).

At first reproduction, there was no effect of Treatment on the relative abundance of spermatocytes (Fig. 4.2a) and male nutritive phagocytes (Fig. 4.2c), but spermatozoa (Fig. 4.2b) were significantly more abundant in the testes of males from the KH and KL treatments than the KM treatment (Table 4.3; Tukey-Kramer test, $p<0.05$). At second reproduction, the relative abundance of spermatozoa in the KL treatment was ~180% (and that of spermatocytes ~50%) of the KM and KH treatments. However, due to low replication (see Materials and methods, Statistical analysis), the power of my analysis was insufficient to detect statistically significant differences among treatments in the abundance of any cell type in the testes at second reproduction ($1-\beta=0.37$, 0.42, and 0.23 for spermatozoa, spermatocytes, and nutritive phagocytes, respectively). In ovaries, nutritive phagocytes (Fig. 4.2d) were significantly more abundant in the KM and KH treatments than the KL treatment at first reproduction (Table 4.3; Tukey-Kramer test, $p<0.05$). At second reproduction, nutritive phagocytes were significantly more abundant in the immature ovaries from the NF treatment than in the mature ovaries from all other treatments (Tukey-Kramer test, $p<0.05$). There was an effect of Aquarium on spermatocytes and male nutritive phagocytes at first reproduction, and an effect of Individual on the relative abundance of each cell type at both reproductive periods (Table 4.3).

Absolute areas of oocytes (Fig. 4.3a) did not differ significantly among the KM, KH, and KL treatments at first or second reproduction, but were significantly smaller in the NF treatment than all other treatments at second reproduction (Table 4.4; GT2 test, $p<0.05$). Absolute areas of ova (Fig. 4.3b) did not differ significantly among treatments

at first or second reproduction. There were no significant effects of Tier, the interaction of Treatment and Tier, or Individual in any analysis of oocyte or ovum area (Table 4.4).

Growth and survival

The growth in test diameter during the 22-month experiment differed markedly among the four treatments (Fig. 4.4). On average, test diameter of sea urchins increased by 320% (34 mm) in the KM treatment, 260% (24 mm) in the KH treatment, and 170% (7 mm) in the KL treatment, but decreased by 4% (0.6 mm) in the NF treatment. Differences in mean test diameter among treatments were highly significant at both first and second reproduction (Table 4.5). There was a significant interaction between Treatment and Tier for test diameter at first reproduction due to small (<2 mm) and inconsistent differences in the middle tier in the KM and KL treatments. Given the power of this analysis to detect small effects, I consider this to be a spurious result.

Survival from the beginning of the experiment to first reproduction, and from first to second reproduction (Fig. 4.5) was similar in the KM, KH and KL treatments (95-100%) but significantly lower (77 and 43%, respectively) in the NF treatment (first reproduction: $F_{3,20}$=8.61, p<0.001; second reproduction: $F_{3,4}$=26.7, p=0.004; SNK, p<0.05).

Feeding rate

The mean feeding rate on kelp (Fig. 4.6) differed significantly among treatments on all three dates (October 1995: $F_{2,15}$=19.6, p<0.001; first reproduction: $F_{2,15}$=139, p<0.001; second reproduction: $F_{2,3}$=20.7, p=0.0176). It was highest in the KL treatment and lowest in the KM treatment in October 1995 and at first reproduction, and significantly higher in the KL treatment than in the KM and KH treatments at second

reproduction (SNK test, $p<0.05$). In October 1995, feeding rate on kelp also differed significantly over a 6-day period in the KM treatment (Fig. 4.7): the rate was lowest on the first day after mussels had been provided and peaked on the third and fourth day, after which it remained similar ($F_{5,30}=21.40$, $p<0.001$; SNK, $p<0.05$).

Table 4.1. Results of nested factorial analysis of variance of gonad index at first and second reproduction. Factors are Feeding Treatment (T), Tier (L), Aquarium (A), and Sex (S). [$^{*}p<0.05$; $^{***}p<0.001$]

Source	*df*	*F*	*p*
First reproduction			
T	2, 4	573.73	<0.001***
L	2, 4	0.69	0.554
T x L	4, 9	0.26	0.894
A (T x L)	9, 155	2.08	0.035*
S	1, 155	0.96	0.328
S x T	2, 155	0.80	0.450
Second reproduction			
T	2, 3	369.78	<0.001***
A (T)	3, 45	0.75	0.529
S	1, 45	67.44	<0.001***
SxT	2, 45	2.60	0.085

Table 4.2. Results of factorial analysis of variance comparing gonad index between Periods (P), Feeding Treatments (T), and Sexes (S). [$^{***}p<0.001$]

Source	*df*	*F*	*p*
P	1, 218	198.78	<0.001***
T	2, 218	320.04	<0.001***
S	1, 218	25.10	<0.001***
P x T	2, 218	2.47	0.087
P x S	1, 218	16.75	<0.001***
T x S	2, 218	0.53	0.591
P x T x S	2, 218	1.14	0.323

Table 4.3. Results of nested factorial analysis of variance of the relative area (percentage of total acinal area) of spermatocytes, spermatozoa, and male and female nutritive phagocytes at first and second reproduction. Factors are Feeding Treatment (T), Tier (L), Aquarium (A), and Individual (I). [$^{*}p<0.05$; $^{**}p<0.01$; $^{***}p<0.001$]

Source	*df*	Spermatocytes		Spermatozoa	
		F	*p*	*F*	*p*
First reproduction					
T	2, 4	3.15	0.149	7.06	0.046*
L	2, 4	0.19	0.834	1.60	0.306
T x L	4, 9	0.61	0.666	0.36	0.831
A (T x L)	9, 16	2.80	0.035*	1.32	0.300
I (T x L x A)	16, 169	9.10	<0.001***	6.68	<0.001***
Second reproduction					
T	2, 3	5.63	0.096	4.81	0.116
A (T)	3, 6	0.19	0.899	0.38	0.774
I (T x A)	6, 60	25.08	<0.001***	32.34	<0.001***

Table 4.3 (continued)

Source	Male nutritive phagocytes			Female nutritive phagocytes		
	df	*F*	*p*	*df*	*F*	*p*
First reproduction						
T	2, 4	1.81	0.275	2, 5	9.95	0.023*
L	2, 4	0.57	0.605	2, 4	5.19	0.070
T x L	4, 9	0.90	0.502	4, 10	0.32	0.858
A (T x L)	9, 16	3.21	0.021*	9, 17	0.63	0.754
I (T x L x A)	16, 169	4.59	<0.001***	17, 174	60.45	<0.001***
Second reproduction						
T	2, 3	2.63	0.220	3, 1	573.56	0.014*
A (T)	3, 6	1.58	0.290	3, 8	0.77	0.542
I (T x A)	6, 60	4.62	<0.001***	8, 75	3.00	0.006**

Table 4.4. Results of nested factorial analysis of variance of absolute areas of oocytes and ova at first and second reproduction. Factors are Feeding Treatment (T), Tier (L), and Individual (I). [$^{***}p<0.001$]

Source	Oocytes			Ova		
	df	*F*	*p*	*df*	*F*	*p*
First reproduction						
T	2, 4	0.02	0.981	2, 4	2.34	0.212
L	2, 4	1.21	0.387	2, 5	0.19	0.831
T x L	4, 8	2.42	0.130	4, 5	0.98	0.492
I (T x L)	8, 97	1.49	0.169	5, 42	1.89	0.117
Second reproduction						
T	3, 6	39.70	<0.001***	2, 1	26.45	0.458
I (T)	4, 44	0.16	0.956	1, 19	0.19	0.665

Table 4.5. Results of nested factorial analysis of variance of test diameter at first and second reproduction. Factors are Feeding Treatment (T), Tier (L), and Aquarium (A). [$^{*}p<0.05$; $^{***}p<0.001$]

Source	*df*	*F*	*p*
First reproduction			
T	3, 6	373.90	<0.001***
L	2, 6	0.41	0.680
T x L	6, 13	4.75	0.010*
A (T x L)	12, 453	0.80	0.650
Second reproduction			
T	3, 4	1091.42	<0.001***
A (T)	4, 86	0.55	0.697

Fig. 4.1. Mean gonad index (+SE) of female, male and unsexed juvenile sea urchins (Juv) immediately prior to the experiment, and adult sea urchins at first (1R) and second (2R) reproduction. Feeding treatments are kelp plus mussel flesh (KM), high ration of kelp (KH), low ration of kelp (KL), and no additional food (NF). Means are based on measurements pooled over all levels of Treatment, Tier (first reproduction only), and Aquarium. Sample sizes are 20 for juveniles prior to the experiment, 25-33 (except 4 unsexed in KL) at first reproduction, and 7-14 (except 3 females in NF) at second reproduction.

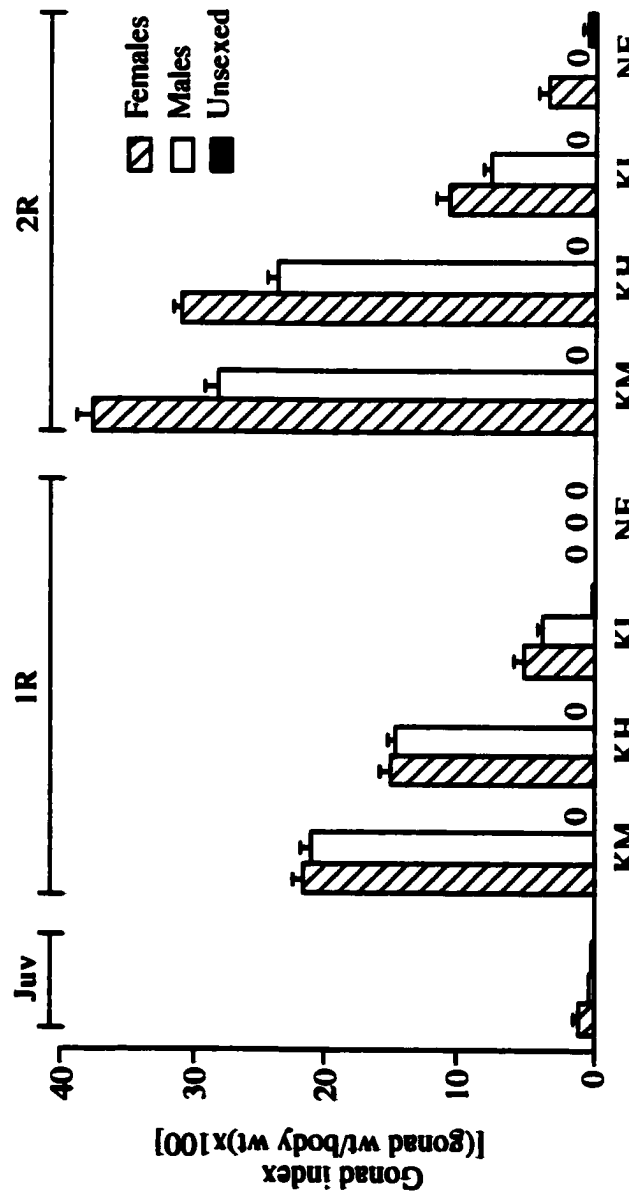
Gonad index
[(gonad wt/body wt)x100]
40
30
20
10
0
Juv
1R
2R
Females
Males
Unsexed
KM
KH
KL
NF
KM
KH
KL
NF
0 0 0
0
0
0
0
0
0
Treatment

Figure 4.1

Fig. 4.2. Mean relative abundance (percentage of cross-sectional area of gonadal acini +SE) of a) spermatocytes, b) spermatozoa, c) male nutritive phagocytes, and d) female nutritive phagocytes in sea urchins from four feeding treatments at first and second reproduction (see Fig. 4.1 for abbreviations). Means are based on measurements pooled over all levels of Treatment, Tier (first reproduction only), Aquarium and Individual (5-6 measurements each). Sample sizes are 60-72 at first reproduction, and 24 (18 for female nutritive phagocytes in NF) at second reproduction.

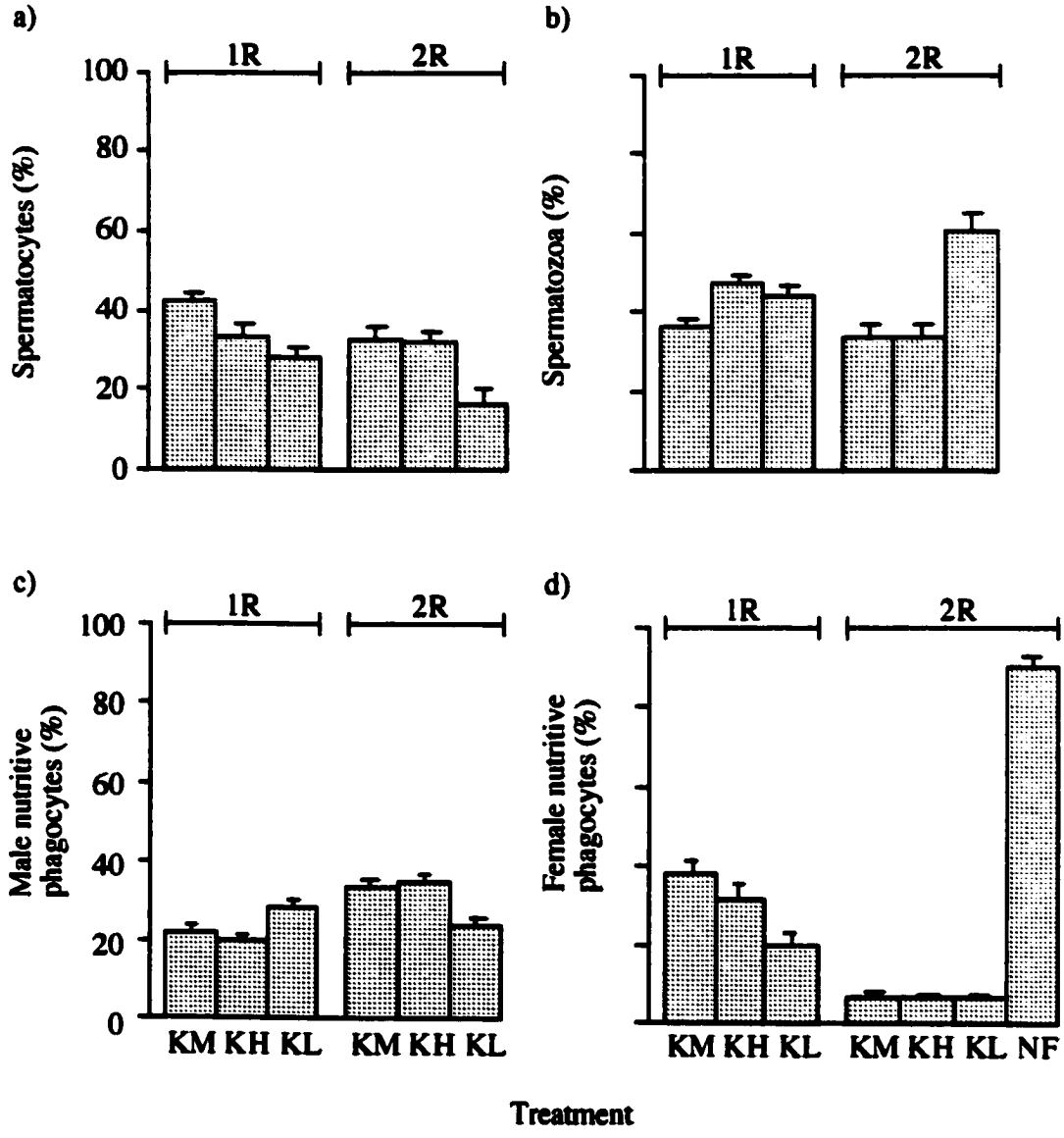
a)
b)
c)
d)
1R
2R
Spermatocytes (%)
Spermatozoa (%)
Male nutritive phagocytes (%)
Female nutritive phagocytes (%)
0
20
40
60
80
100
KM KH KL KM KH KL
KM KH KL KM KH KL NF
Treatment

Figure 4.2

Fig. 4.3. Mean (+SE) absolute areas of a) oocytes and b) ova of female sea urchins from four feeding treatments at first and second reproduction (see Fig. 4.1 for abbreviations). Means are based on mean gamete areas in 1-4 gonadal acini per female and are pooled over all levels of Treatment, Tier (first reproduction only), Aquarium and Individual. Sample sizes are 35-42 (mean oocyte area) and 9-25 (mean ovum area) at first reproduction, and 6-16 (mean oocyte area) and 8-11 (mean ovum area) at second reproduction.

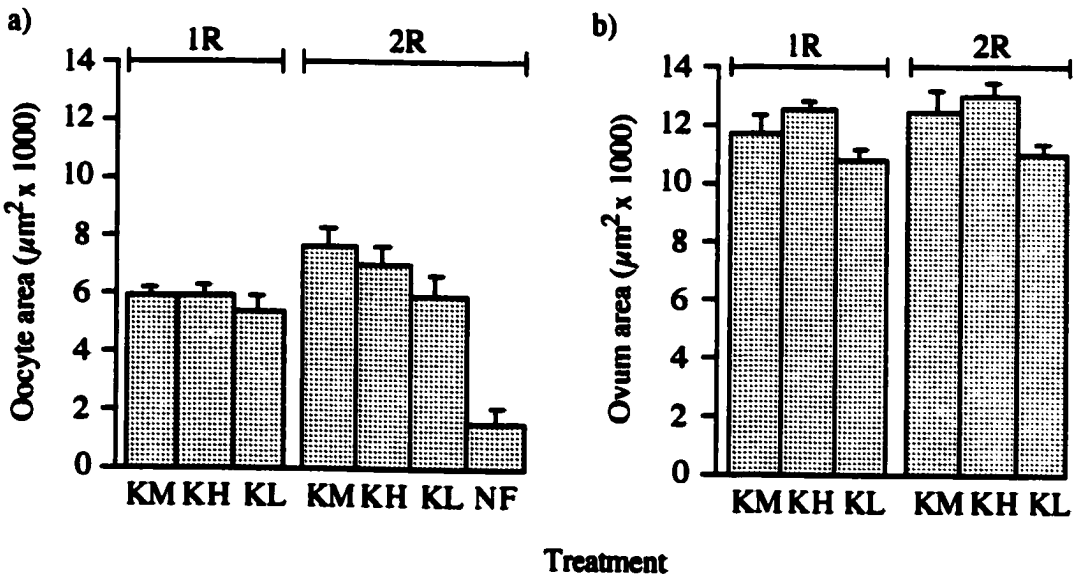
a)
1R
2R
14
12
10
8
6
4
2
0
Oocyte area (μm² x 1000)
KM KH KL KM KH KL NF
b)
1R
2R
14
12
10
8
6
4
2
0
Ovum area (μm² x 1000)
KM KH KL KM KH KL
Treatment

Figure 4.3

Fig. 4.4. Mean test diameter (±SE, often hidden by symbols) of sea urchins from four feeding treatments at multiple times during the experiment (see Fig. 4.1 for abbreviations). Means are based on measurements pooled over all levels of Treatment, Tier (May to March 1996), and Aquarium. Sample sizes are 98-130 for May 1995 to March 1996, and 17-40 for April 1996 to March 1997.

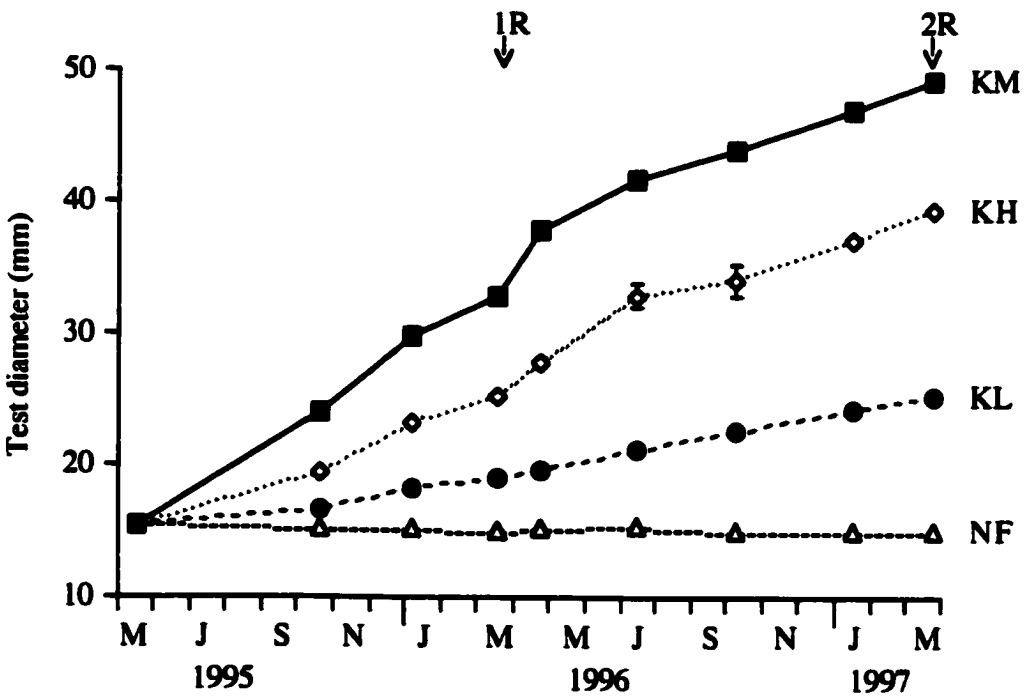
1R
2R
50
40
30
20
10
Test diameter (mm)
KM
KH
KL
NF
M J S N J M M J S N J M
1995
1996
1997

Figure 4.4

Fig. 4.5. Mean survival (+SE) of sea urchins from four feeding treatments to first and second reproduction (see Fig. 4.1 for abbreviations). Means are based on 6 (first reproduction) or 2 (second reproduction) replicate aquaria per treatment.

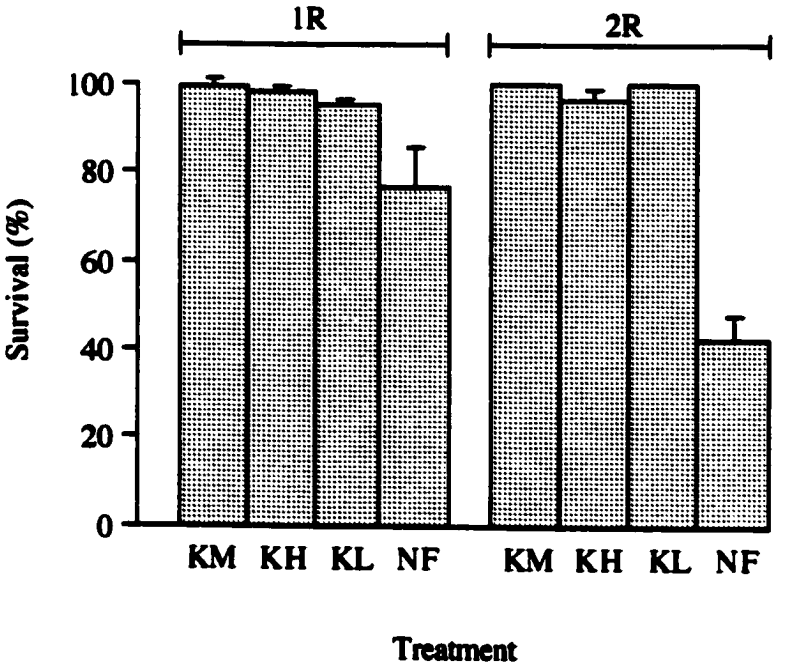

Figure 4.5

Fig. 4.6. Mean feeding rate (+SE) of sea urchins from three feeding treatments in October 1995, at first and second reproduction (see Fig. 4.1 for abbreviations). Means are based on 6 (October 1995, first reproduction) or 2 (second reproduction) replicate aquaria per treatment.

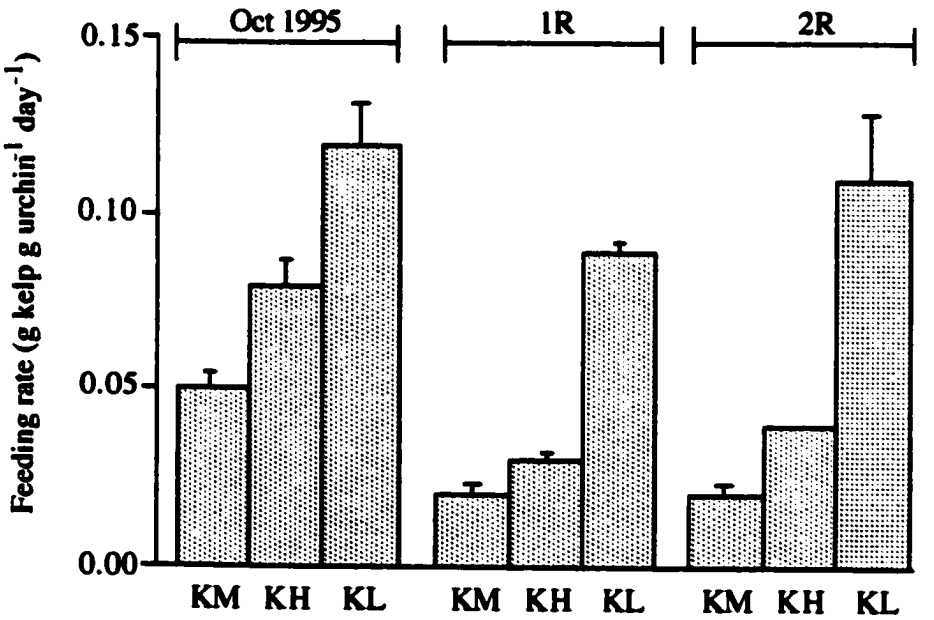
Oct 1995
1R
2R
0.15
0.10
0.05
0.00
Feeding rate (g kelp g urchin^{-1} day^{-1})
KM KH KL KM KH KL KM KH KL
Treatment

Figure 4.6

Fig.4.7. Mean feeding rate (+SE) of sea urchins in the KM treatment over six days of feeding on kelp in October 1995. Means are based on 6 replicate aquaria.

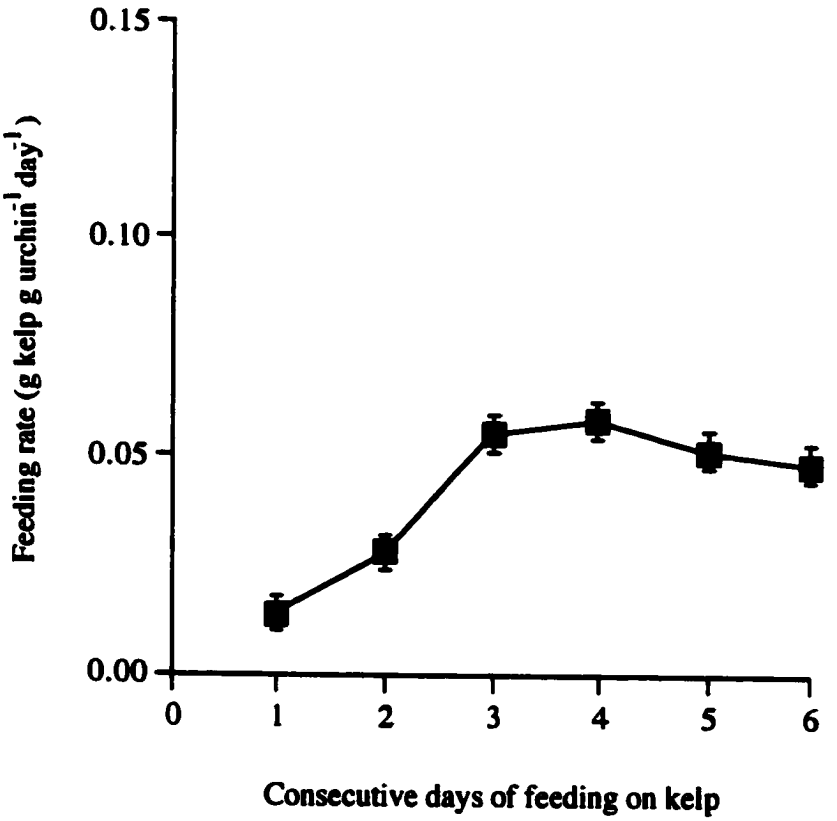
0.15
0.10
0.05
0.00
Feeding rate (g kelp g urchin^{-1} day^{-1})
0
1
2
3
4
5
6
Consecutive days of feeding on kelp

Figure 4.7

DISCUSSION

Reproduction

Nearly all *Strongylocentrotus droebachiensis* fed a high food ration and most (83%) fed a low ration became reproductively mature in the first year of the experiment. Sea urchins grazing only on encrusting coralline algae did not mature during the experiment, delaying their first reproduction by at least two years. A positive effect of food supply on age at first reproduction has also been reported for populations of *S. droebachiensis* occurring in different habitats in Norway (Sivertsen and Hopkins 1995), and *Echinocardium cordatum* in England (Buchanan 1966). Other factors affecting the allocation of energy to reproduction and/or growth, such as energy expenditure for foraging, predator avoidance, and maintenance, often vary among habitats (Ebert 1968, Mattison et al. 1977, Harrold and Reed 1985), thus confounding the potential effects of food availability. To my knowledge, this study is the first to show that diet strongly affects reproductive maturation of juvenile sea urchins when other confounding factors are controlled.

In my laboratory study, diets of high food quality and quantity (KM, KH) greatly enhanced gonad growth in *S. droebachiensis*. This result is consistent with differences in gonad index recorded in field populations with varying food supply, e.g., kelp beds and barrens (Lang and Mann 1976, Vadas 1977, Chapter 2). At first reproduction, the gonad index of sea urchins fed either kelp and mussels (21%) or a high ration of kelp (15%) was markedly higher than the gonad index in natural populations (3%; Raymond and Scheibling 1987, Munk 1992). The gonad index of sea urchins fed a low ration of kelp (5%) was comparable to field values. The gonad index recorded at second reproduction in female sea urchins fed a diet of kelp and mussels (38.3%) is, as far as I am aware, the highest reported to date for *S. droebachiensis*. De

Jong-Westman et al. (1995a) give an index of 39% based on drained body mass (~34.5% based on total body mass, i.e., with coelomic fluid) for *S. droebachiensis* fed a high-protein diet (supplemented with β-carotenes) in the laboratory. The index I recorded is considerably higher than maximum indices reported for adults of *S. droebachiensis* in other laboratory studies (23-27%; Vadas 1977, Larson et al. 1980, Minor and Scheibling 1997) or in natural habitats with abundant macroalgal food (17-34%; Cocanour and Allen 1967, Himmelman 1975 1978, Vadas 1977, Lee and Haard 1982, Keats et al. 1984, Munk 1992, Chapter 2). My results and those of De Jong-Westman et al. (1995a) demonstrate that a protein addition to the diet enables *S. droebachiensis* to grow larger gonads than a diet consisting of macroalgae only. Other laboratory or field studies also found a positive influence of protein on gonadal growth in *Strongylocentrotus intermedius* (Levin and Naidenko 1987), *Paracentrotus lividus* (Lawrence et al. 1992), and *Echinus esculentus* (Emson and Moore 1998).

At first reproduction, females and males within a treatment had similar gonad indices, while at second reproduction, females had significantly higher gonad indices. A sexual difference in gonad index in large sea urchins has previously been demonstrated in *S. droebachiensis* (Munk 1992, de Jong-Westman et al. 1995a, Minor and Scheibling 1997, Chapter 2) but not other congeneric species (Bennett and Giese 1955, Fuji 1960b, Bernard 1977). The absence of a sexual difference in smaller *S. droebachiensis* also was reported by Munk (1992) and may be related to a limited allocation of resources to reproduction in small adults (Thompson 1979, 1984, Lawrence 1987).

The significant increase in mean gonad index from first to second reproduction in all fed treatments suggests a greater energy allocation to reproduction with increasing body size or age (Thompson 1979, 1984, Lawrence 1987). This is consistent with previous studies of *S. droebachiensis* (Munk 1992, Chapter 2) and *S. purpuratus*

(Gonor 1972) which show that gonad index increases exponentially in small sea urchins and reaches an asymptote in larger individuals. Because of this pattern, comparisons of gonad index among my different feeding treatments were complicated as unfed animals and those on a low ration of kelp were smaller and would therefore have a lower gonad index, irrespective of diet, than the larger sea urchins fed kelp and mussels or a high ration of kelp. Nevertheless, marked differences between treatments in gonad mass were apparent upon dissection. Gonads of sea urchins fed kelp and mussels completely filled the test, while gonads of sea urchins fed kelp only occupied considerably less space, and those of unfed sea urchins were barely visible.

Although diet had a large effect on gonad size, its effect on gametogenesis in *S. droebachiensis* was relatively small. The relative abundance of different cell types in gonads, and the absolute sizes of oocytes or ova generally were comparable among sea urchins that received macroalgal food, with or without a protein supplement. This is consistent with findings of Minor and Scheibling (1997) for a similar feeding experiment, and my own findings (Chapter 2) for natural populations of *S. droebachiensis* with differing food supplies. Males fed kelp and mussels, however, had proportionally more spermatocytes and less spermatozoa in testes than males fed only kelp, at either ration. I attribute this to a sampling artifact: because some males fed kelp and mussels were too mature to process (disintegrated upon dissection), my sample was biased in favour of less mature males (see also Minor and Scheibling 1997). At second reproduction, females in the unfed treatment had significantly smaller oocytes than females in other treatments, reflecting their immaturity.

Growth and survival

The growth rate of *Strongylocentrotus droebachiensis* differed markedly among feeding treatments, from 16-18 mm per year for sea urchins fed kelp and mussels, to 10-14 mm for those on a high ration of kelp, to only 3-7 mm for those on a low ration of kelp. These growth rates are within the range of those previously recorded for small (8-16 mm) *S. droebachiensis* in the field (3-17 mm per year; Swan 1961, Miller and Mann 1973, Lang and Mann 1976, Himmelman 1986, Himmelman et al. 1983a, Raymond and Scheibling 1987, Chapter 3) but generally lower than the growth rate observed in another laboratory study (15-31 mm per year, Vadas 1977). Growth rates in fed sea urchins were approximately linear during the study period as the animals were approaching an asymptotic test diameter (~50 mm; Chapter 3). The lack of growth in sea urchins grazing on coralline algae is consistent with previous suggestions that the slow growth rate of juveniles of *S. droebachiensis* on some barrens (1-2 mm per year) results from severe food shortage (Himmelman et al. 1983b, Keats et al. 1985, Himmelman 1986).

Survival rate of sea urchins in the treatment provided with only coralline algae (77% to first reproduction, and 43% to second) was significantly lower than in the other feeding treatments (95-100%) suggesting that animals without added food were starving to death. Raymond and Scheibling (1987) also found significantly reduced survival in juveniles of *S. droebachiensis* fed encrusting coralline algae only (87%) relative to those fed kelp and corallines (99%).

Tier and Aquarium effects

There was no significant effect of Tier in any of the analyses, and only one significant interaction between Tier and Treatment (in the analysis of test diameter at first

reproduction) which I have dismissed as spurious. The effect of Aquarium was significant in analyses of gonad index (Table 4.2), and male nutritive phagocytes and spermatocytes (Table 4.4) at first reproduction. The term Aquarium enables detection of a potential effect of the positions of replicate aquaria within each Treatment by Tier combination. Further analysis showed that the highest and lowest aquarium means in each of the three cases where the Aquarium effect was significant occurred in the bottom tier in both the KH and KL treatments (there was no apparent pattern in these means in the KM treatment). I could not detect any consistent differences (e.g., in temperature, water supply, or lighting) between aquaria in the bottom tier which might explain this statistical result.

Feeding rate

Sea urchins fed once a week had a higher feeding rate than sea urchins fed continuously, which is consistent with results from a similar experiment with adult *Strongylocentrotus droebachiensis* (Minor and Scheibling 1997). A functional response to varying food supply allows sea urchins in a food-limited environment to exploit a temporarily abundant food source, and thus maximize energy intake. Feeding rates in each treatment were similar at first and second reproduction but declined from fall to spring. A seasonal decrease in feeding rate in connection with gonad maturation has previously been demonstrated for *S. droebachiensis* (Vadas 1977, Himmelman 1980, Keats et al. 1983, Himmelman and Nédélec 1990, Chapter 2) and congeneric species (Fuji 1962, Lawrence et al. 1965, Ebert 1968, Vadas 1977, Kawamata 1997). Sea urchins fed kelp and mussels always had a lower feeding rate on kelp than sea urchins fed kelp only, suggesting that an addition of protein to the diet decreases the overall amount of food consumed. This is supported by the reduction in feeding rate observed

in the KM treatment in the two days following the provision of mussel flesh compared to later days.

Conclusions

My experiment indicated that *Strongylocentrotus droebachiensis* fed a high ration of high quality food produce large gonads at first reproduction. Sea urchins fed a lower ration may delay reproduction by one year, and produce significantly less gonadal material. Sea urchins on a low quality and quantity diet, coralline algae, may not survive to reproduce, and survivors delay reproduction by at least two years. These results are consistent with the findings of Sivertsen and Hopkins (1995) in Norway that *S. droebachiensis* reproduces at a younger age in kelp beds than in barrens. As young sea urchins are abundant in both these habitats (Chapter 3), a shift in age at first reproduction would greatly influence the proportion of reproductive individuals. Higher adult density and greater reproductive output at first reproduction increase total population reproductive output, which is advantageous in free-spawners such as *S. droebachiensis* where higher sperm concentrations enhance fertilization success (Pennington 1985, Denny and Shibata 1989, Levitan 1991, Grosberg 1991, Oliver and Babcock 1992, Yund 1995). Because of the greater reproductive value of young individuals, larval production by a given number of sea urchins may thus be increased in kelp beds relative to barrens to a greater extent than indicated by differences in gonad index of older adults alone. The reproductive potential of young sea urchins must be considered when assessing the importance of subpopulations of *Strongylocentrotus droebachiensis* in different habitats as contributors to the overall larval pool, and hence in the outbreak dynamics of this species along the Atlantic coast of Nova Scotia. The delayed maturation and low juvenile survival I detected in the treatment with only

coralline algae suggests that sea urchins in severely food-limited environments (e.g., deep-water barrens) may have little influence on the overall population dynamics of this species.

The decline of the fishery for *Strongylocentrotus* spp. on both coasts of North America in recent years (Rogers-Bennett et al. 1995, McLaughlin et al. 1996, Creaser and Hunter 1997) and the periodic elimination of *S. droebachiensis* populations due to disease along the Atlantic coast of Nova Scotia (Miller 1985a, Miller and Colodey 1983, Scheibling 1986, Scheibling and Hennigar 1997) have stimulated interest in aquacultural and sea-ranching techniques (Hatcher and Hatcher 1997). One promising approach is to collect small individuals from natural habitats where they are abundant (e.g., barrens) and grow them to commercial size (52 mm) in land or sea-based facilities. My study demonstrated that juveniles of *S. droebachiensis* (15 mm) can reach marketable size with very large roe yields in approximately two years when fed a protein-enriched diet. This information can be profitably used by industry to develop aquacultural practices that benefit from an abundant and a previously unrealized resource.

Chapter 5: Relative importance of parental and larval nutrition on larval development and metamorphosis

INTRODUCTION

Larval survival and development can have a pronounced effect on the rates of settlement and recruitment, and hence on the distribution, demography, and dynamics of adult populations of benthic marine invertebrates (Scheltema 1986, Grosberg and Levitan 1992, Balch and Scheibling in press). To a great extent, rates of survival and development of larvae are determined, either directly or indirectly, by larval nutritional condition. For planktotrophic larvae, it is well established that food quality and quantity have a direct positive effect on larval condition, manifested by increased survival and growth, and by accelerated development and metamorphosis (e.g., Gastropoda: Aldana Aranda et al. 1989; Bivalvia: Strathmann et al. 1993; Decapoda: Anger 1984; Asteroidea: Lucas 1982; Echinoidea: Hart and Strathmann 1994; Holothuroidea: Martinez and Richmond 1998). Maternal reproductive characteristics such as fecundity, egg size, and egg quality also can be affected by food quality and quantity (e.g., Bayne et al. 1978, Thompson 1982, George et al. 1990, 1991, Qian 1994, Lucas and Lawes 1998). However, because planktotrophic larvae obtain nearly all of their energy from sources other than the egg, possible effects of maternal condition (as manifested by egg size or quality) on larval development have been largely ignored in this group. In the few studies which have addressed this issue, maternal condition was shown to influence characteristics of planktotrophic larvae such as size, chemical composition and survival rate; however, when present, these effects were small or did not persist throughout the developmental period (e.g., George 1990, George et al. 1990, de Jong-Westman et al.

1995b, Guisande and Harris 1995, Bertram and Strathmann 1998, Lucas and Lawes 1998). The relative importance of parental condition and larval feeding on larval survival and development remains largely unknown (but see Bertram and Strathmann 1998).

Strongylocentrotus droebachiensis is the dominant herbivore in the shallow rocky subtidal zone in the northwest Atlantic, where it undergoes periodic population outbreaks leading to the destruction of kelp beds and formation of sea urchin-dominated barren grounds (Mann 1977, Wharton and Mann 1981, Chapter 1). Following this transition, gonadal production of sea urchins decreases in barrens with the reduction in macroalgal food availability (Lang and Mann 1976, Johnson and Mann 1982, Chapter 2). Differences in adult nutrition which affect fecundity also may influence egg quality in sea urchins from kelp beds and barrens. Recent studies of *S. droebachiensis* have shown that larvae from parents raised on different artificial diets in the laboratory (de Jong-Westman et al. 1995b), or collected from different natural habitats (Bertram and Strathmann 1998), differ in their rates of development, growth and survival.

In this study, I investigate the relative importance of parental nutritional conditioning and larval food ration on the size and morphology of larvae of *S. droebachiensis*, and their rates of development, survival and metamorphosis, in a laboratory experiment. I also measure the size of juveniles shortly after settlement to determine whether differences in larval quality persist beyond metamorphosis. To circumvent the confounding of geographic (genetic) variability and adult nutritional condition (e.g., George 1990, George et al. 1990, Bertram and Strathmann 1998), I use juveniles from a single source population and rear them to reproductive maturity under tightly controlled conditions. Because food quality and quantity varies widely between adult populations in kelp beds and barrens (Chapters 2, 3), any effect of parental nutrition on larval survival and metamorphosis may influence the overall reproductive

success of this species. Understanding the potential importance of parental condition in determining larval quality may shed light on sea urchin recruitment patterns and outbreak dynamics.

MATERIALS AND METHODS

Experimental design

To investigate the simple and interactive effects of parental conditioning and larval nutrition on larval development and metamorphosis of *Strongylocentrotus droebachiensis*, I conducted a 2 x 2 factorial experiment in the laboratory. To attain different levels of parental conditioning, I collected juvenile sea urchins (horizontal test diameter: 13-17 mm) from a barren ground at 6-8 m depth off Little Duck Island (see Chapter 2 for a description of the collection site), and reared them to reproductive maturity as part of a broader 22-month (May 1995 to March 1997) feeding experiment (see Chapter 4 for details of the experiment). Feeding treatments used in the present study are 1) KM, a high ration of kelp (blades of *Laminaria longicruris* and *L. digitata* for 6 d wk^{-1}) augmented with mussel flesh (*Mytilus edulis* and *M. trossulus* for 1 d wk^{-1}), and 2) KL, a low ration of kelp (1 d wk^{-1}). At the peak of their second reproductive season (March 1997), sea urchins of both sexes in the KM treatment had a significantly higher mean gonad index (expressed as a percentage of total wet body weight) than those in the KL treatment (females: 38% vs. 11%, males: 28% vs. 8%) (Chapter 4). Sea urchins in the KM treatment also were significantly larger (49 mm) than those in the KL treatment (25 mm). Mean egg size (as cross-sectional area, μm^2), however, did not differ significantly between the two treatments (KM, 1.25×10^4; KL, 1.10×10^4).

On March 20, 1997, I induced spawning in ten randomly selected sea urchins from each of the two parental treatments by coelomic injection of 0.5-1.5 ml of 0.53*M*

KCl. Females spawned into glass beakers containing ~120 ml of chilled, 0.45 μm Millipore®-filtered seawater (hereafter referred to as filtered seawater) and males spawned into dry, chilled trays. After ensuring that female spawn contained only mature eggs, I washed it three times with filtered seawater. I checked dry sperm from three males for motility and mixed it in a chilled tray. I collected sperm by inserting the tip of a clean spatula into the mixture and then added it to the eggs of individual females. After stirring the egg-sperm mixtures gently for 10 min, I rinsed the eggs three times with filtered seawater to remove excess sperm. Fertilization rates of individual females were calculated as the percentage of 100 eggs showing an elevated perivitelline membrane. The fertilized eggs of the three females with the highest fertilization rates (mean ±SE: KM, 94.0 ±0.9%; KL, 98.7 ±0.6%) then were mixed and distributed among six glass finger bowls containing 135 ml of filtered seawater, to form a monolayer in each bowl.

I reared embryos for 48 h in finger bowls at 12°C before transferring them to 12 (6 each for the KM and KL treatments) 4 l glass jars containing 2 l of filtered seawater. For the remainder of the experiment, larvae were kept at 9°C under a 12L:12D photoperiod at a light intensity of 39.4 ±2.9 μEin m^{-2} s^{-1} (mean ±SD, n=3) (hereafter referred to as standard culture conditions). Cultures were stirred with T-paddles rotating at 10 rpm. At the start of the experiment, larval density in culture jars was ~1 larva ml^{-1}. I randomly assigned three replicate jars from each parental treatment (KM, KL) to either a high ration (5000 cells ml^{-1}) or a low ration (500 cells ml^{-1}) of the green alga *Dunaliella tertiolecta*, grown in f/2 nutrient medium under constant fluorescent illumination at 23°C. Every 2 d, I replaced 60-70% of the culture water with freshly filtered seawater and, starting at 4 d after fertilization when larvae were in the late prism stage, added exponentially growing algae. Cultures of the high ration treatment were terminated at 39 d when most larvae had settled. Cultures of the low ration treatment were terminated at

55 d when development had effectively ceased and only few larvae had reached competency. I chose larval food rations and the temperature regime for consistency with Hart and Scheibling (1988a).

Larval development and morphology

At 6, 12, 19, 22, 27, and 33 d after fertilization for all treatment combinations, and at 55 d for the low ration treatment only, I removed 8-29 larvae from each jar and videotaped them on glass microscope slides using a Hi8 camera (Panasonic WV-3170A) and tapedeck (Sony EV-S900 NTSC) connected to a binocular microscope (Leitz Labovert). I analysed video records using an image analysis system (NIH *Image*, Version 1.60; National Institutes of Health, Bethesda, Maryland, USA). I classified larvae as being in the 4, 6, or 8-arm developmental stage. When first analysed (at 6 d), all larvae were clearly in the 4-arm stage (with arm lengths >165 and >80 μm for the 1st and 2nd arm pair, respectively). I considered larvae as having attained the 6 or 8-arm stage when the 3rd or 4th arm pair, respectively, exceeded a length of 25 μm. For each replicate jar, I calculated the frequency of each developmental stage as a percentage of the total number of larvae sampled.

For measurements of growth and morphology, I analysed subsamples of 6-10 larvae per jar at each sampling date. I measured the length of the postoral, anterolateral, posterodorsal and preoral arms from the base to the arm tip; the body length from the posterior tip to the transverse ciliary band between the postoral arms; and the body width at the base of the postoral arms or between the posterior epaulettes once they had developed.

To record the decline in larval number over time (due to mortality or metamorphosis), I counted larvae in 3-4 replicate subsamples of 5 ml from each jar at 2 (upon transfer to the culture jars), 22, 39, and 55 d.

Metamorphosis and size of settlers

At 33 and 36 d after fertilization (high ration treatment) or 55 d (low ration treatment), I induced metamorphosis of larvae in the 8-arm stage with a well-developed rudiment. I pipetted 30 (high ration) or 15 (low ration) larvae from each culture jar into each of three finger bowls containing 100 ml of filtered seawater. Each bowl contained one pebble (~2 cm in diameter) collected from a nearby subtidal site and encrusted with coralline algae (*Phymatolithon laevigatum*, *Lithothamnion glaciale*), which are known to induce metamorphosis in *Strongylocentrotus droebachiensis* (Pearce and Scheibling 1990, 1991). Before use in the induction trials, I scrubbed pebbles to remove any adhering organisms. I calculated the rate of metamorphosis after 24 h as the number of settlers expressed as a percentage of the total number of individuals (larvae and settlers) retrieved (on average, >98% were retrieved), and videotaped settlers (on glass slides) for measurement of test diameter. All settlers from replicate bowls from each culture jar were then pooled in a clean glass bowl and kept for one week without added food under standard culture conditions before being videotaped again.

Statistical analysis

To determine the effects of parental conditioning and larval food ration on larval morphology, I used principal components analysis (PCA) based on measurements of arm length (mean of two measurements for each of four arm pairs), body length and body width. All measures were log transformed, and a small constant (1) was added to

allow inclusion of zero lengths for preoral arms in larvae fed the low ration. For PCA, I used the correlation (rather than covariance) matrix because some measurements (preoral arm lengths) differed by two orders of magnitude between larvae from the high and low rations (Reyment et al. 1984). I conducted three separate analyses to compare fully developed larvae (at 33 d for the high ration or at 55 d for the low ration) between parental conditioning treatments within the high and the low larval ration (PCA 1 and 2, respectively), and between parental and larval treatment combinations (PCA 3). For each larva included in a PCA, I calculated the mean score of the six characters measured for the first (PC 1) and second (PC 2) principal components.

I used two or three-way analysis of variance (ANOVA) to compare mean principal component scores among larvae in the different parental conditioning and/or larval ration treatments. Parental Conditioning (KM, KL) and Larval Ration (high, low) were fixed factors, and Jar (3 levels) was a random factor nested within Parental Conditioning (two-way ANOVA) or within the interaction of Parental Conditioning and Larval Ration (three-way ANOVA). In each analysis, there were 10 replicate mean scores (9 in one case) per jar. Despite log-transformation, variances remained heterogeneous (as shown by Cochran's C test at α=0.05) in PCA 2 and 3. I maintained the factor designation, factor levels, and nesting terms used here in all other analyses.

I compared the percentage of larvae remaining at 39 d (relative to the number transferred to culture jars at 2 d) between treatments using two-way ANOVA, with the factors Parental Conditioning and Larval Ration, and three replicate measures (means of 3–4 counts per jar) per treatment combination. I compared the percentage of larvae remaining at 55 d between parental treatments in the low ration with a t-test.

I analysed differences in the rate of metamorphosis between treatments using counts of larvae and settlers transformed using the following equation (Zar 1984):

$$\eta' = \sqrt{(h+0.5)}\arcsin\sqrt{\left(\frac{q+0.375}{h+0.75}\right)} \qquad [5.1]$$

where η' is the transformed rate, h is the number of retrieved larvae and settlers, and q is the number of retrieved settlers. This transformation is appropriate when data are at the extreme ends of the range of possible values (Zar 1984). I measured the rate of metamorphosis twice in the high ration and once in the low ration. For the high ration and for each parental treatment, two-way ANOVA with the random factors Trial (2 levels) and Jar, and 3 replicate bowls per jar, showed that there was no significant difference ($p>0.05$) in the rate of metamorphosis between the first and second trials. I therefore pooled trials for the high ration to compare rates of metamorphosis among parental and larval treatment combinations using three-way ANOVA with the factors Parental Conditioning, Larval Ration, and Jar. There were 6 replicates per jar for the high ration (pooled from two trials) and three for the low ration.

I measured horizontal test diameters of settlers 24 h and 1 wk after induction twice in the high ration and once in the low ration. Using the same analysis as for rate of metamorphosis in the high ration, I found no significant difference ($p>0.05$) in the test diameter of settlers after 24 h or 1 wk between trials and thus pooled them. To compare test diameter among parental and larval treatment combinations after 24 h and 1 wk, I used two-way ANOVA with the factors Parental Conditioning and Larval Ration but was unable to test for the factor Jar because of insufficient replication in the low ration (0-3 settlers per jar).

In analyses where replication levels differed among groups, I applied ANOVA techniques for unbalanced data using Type III sums of squares. Because the sums of squares in an unbalanced model are not necessarily independent, the denominator mean square of the F-ratio generally is constructed from a linear combination of mean squares,

based on the variance components. The degrees of freedom for the divisor are estimated using the Satterthwaite approximation. For further discussion of these techniques, see Minor and Scheibling (1997). I used *t*-tests to carry out post-hoc comparisons between means in analyses where interaction terms were significant. To determine the relative importance of each factor in analyses with significant results, I calculated the magnitude of the experimental effect using a fixed model (Howell 1987) which excluded the factor Jar (not significant in these analyses).

RESULTS

Larval development and morphology

The rate of development from the 4-arm to the 8-arm stage was faster in larvae fed a high than a low ration of microalgal food (Fig. 5.1). All larvae fed a high ration reached the 8-arm stage at 27 d after fertilization, while a small percentage of larvae fed the low ration were still at the 6-arm stage at 55 d. Within the low ration, few of the larvae that reached the 8-arm stage had well-developed rudiments. The rate of development generally was similar in the two parental conditioning treatments within each larval food ration (Fig. 5.1), although development to the 8-arm stage was significantly more advanced in the KL than the KM treatment at 19 (*G*-test, χ^2=8.08, p<0.005) and 33 d (χ^2=8.08, p<0.005).

Differences in size-at-age between larval rations reflected the difference in developmental rate: larvae fed the high ration had longer arms and a longer but narrower body than those fed the low ration (Fig. 5.2). At the end of the larval period (at 33 and 55 d for the high and low rations, respectively), body and arm lengths (except posterodorsal arms in the KM treatment) tended to be larger, and body width smaller, in larvae fed the high ration (Fig. 5.2). For each ration, larvae from the two parental

treatments had a similar morphology throughout most of development, with two exceptions. Firstly, larvae fed the high ration were larger in the KL than the KM treatment at the end of the larval period. Secondly, larvae fed the low ration had longer arms in the KL than the KM treatment at 19 and 33 d, corresponding to the more advanced development of larvae in the KL treatment on these dates.

Principal components analysis distinguished between parental and larval treatments on the basis of size and morphology (Fig. 5.3; Table 5.1). In all analyses, the first principal component (PC 1), which explained 51-52% of the overall variance (Table 5.1), described size differences. The larval arms and body (which elongate during development) had high positive coefficients while body width (which becomes smaller) had a low positive or negative coefficient. Therefore, larvae with high mean PC 1 scores were larger and more developed than those with low scores. The second principal component (PC 2), which explained 17-19% of the overall variance, distinguished larvae mainly on the basis of body width, which had a high positive coefficient while all other characters had lower, positive or negative coefficients (Table 5.1). Larvae with high mean PC 2 scores had a wider body, and therefore were less developed, than those with low mean scores.

The ANOVA based on PCA 1, which compared fully developed larvae between parental treatments within the high ration, indicated that larvae were significantly larger in terms of body and arm lengths (PC 1: $F_{1,4}$=10.55, p=0.031) in the KL than the KM treatment, but that they did not differ significantly in body width (PC 2: $F_{1,4}$=0.08, p=0.795). The ANOVA based on PCA 2, which compared fully developed larvae between parental treatments within the low ration, showed that these larvae reached a similar size and morphology regardless of parental conditioning (PC 1: $F_{1,4}$=0.49, p=0.524; PC 2: $F_{1,4}$=0.42, p=0.554). The ANOVA based on PCA 3, which compared

fully developed larvae between parental and larval treatment combinations, showed that larvae fed the high ration were significantly larger, again in terms of body and arm lengths (PC 1: $F_{1,8}$=10.87, p=0.011) but not body width (PC 2: $F_{1,8}$=5.08, p=0.054), than those fed the low ration. There were no significant effects of parental conditioning (PC 1: $F_{1,8}$=0.21, p=0.659; PC 2: $F_{1,8}$=1.09, p=0.327) or of the interaction between parental conditioning and larval ration (PC 1: $F_{1,8}$=3.03, p=0.120; PC 2: $F_{1,8}$=0.06, p=0.813). The random factor Jar was significant in the ANOVA based on PCA 1 for both principal components (PC 1: $F_{4,54}$=5.44, p<0.001; PC 2: $F_{4,54}$=2.67, p=0.042), and in the ANOVA based on PCA 3 for the first component only (PC 1: $F_{8,107}$=2.13 p=0.040; PC 2: $F_{8,107}$=1.30, p=0.250). Jar effects were non-significant in the ANOVA based on PCA 2 (PC 1: $F_{4,53}$=1.85 p=0.134; PC 2: $F_{4,53}$=1.12, p=0.356).

Larval number in cultures declined markedly over time, more so for the KM than the KL treatment, and for the high than the low ration (Fig. 5.4). At 39 d, the percentage of larvae remaining within the high ration was significantly lower in the KM than the KL treatment ($F_{1,8}$=21.73, p=0.002) but there was no significant difference between rations ($F_{1,8}$=2.88, p=0.128) and no significant interaction between parental and larval treatments ($F_{1,8}$=0.03, p=0.876). Analysis of the magnitude of effects showed that parental conditioning accounted for 61.6% of the overall variability in larval numbers, larval ration for 5.6%, and the interaction between these factors for <0.01%. At 55 d, the percentage of larvae remaining within the low ration did not differ significantly between parental treatments (t_4=2.05, p<0.200).

Metamorphosis and size of settlers

ANOVA comparing rates of metamorphosis among treatment combinations (Fig. 5.5) indicated significant effects of parental conditioning ($F_{1,10.3}$=9.00, p=0.013), larval

ration ($F_{1,10.3}$=258, p<0.001), and the interaction between these factors ($F_{1,10.3}$=9.18, p=0.012), but no significant effect of jar ($F_{8,42}$=0.92, p=0.511). Post-hoc tests showed that, for each parental treatment, the rate of metamorphosis was significantly greater within the high than the low ration (KM: t_{25}=12.47, p<0.001; KL: t_{25}=9.43, p<0.001). The rate of metamorphosis also was significantly greater in the KM than the KL treatment within the high ration (t_{34}=4.43, p<0.001), but did not differ significantly between parental treatments within the low ration (t_{16}=0.03, p>0.90). Analysis of the magnitude of experimental effects (excluding jar effects) showed that larval ration explained 75.7% of the variance in the rate of metamorphosis, whereas parental conditioning and the interaction of these two factors explained only 2.3% and 1.8%, respectively.

A comparison of test diameter among all treatment combinations showed that settlers from the high ration were significantly larger than those from the low ration after 24 h ($F_{1,477}$=20.35, p<0.001) and 1 wk ($F_{1,342}$=7.59, p=0.006) (Fig. 5.6). Test diameter did not differ significantly between parental treatments (24 h: $F_{1,477}$<0.01, p=0.959; 1 wk: $F_{1,342}$=0.55, p=0.458) and there was no significant interaction between larval and parental treatments (24 h: $F_{1,477}$=3.82, p=0.051; 1 wk: $F_{1,342}$=0.08, p=0.772). Test diameter remained relatively constant during the first week after settlement in all treatment combinations (Fig. 5.6).

Table 5.1. Results of three principal components analyses (PCA) (see Materials and Methods for explanation of the respective analyses) based on larval arm lengths (POA, postoral arms; ALA, anterolateral arms; PDA, posterodorsal arms; PRA, preoral arms), body length (BLE) and body width (BWI). Data are coefficients and eigenvalues for the first (PC 1) and second (PC 2) principal components, and percentage of variance explained by each.

	Coefficient							
PC	POA	ALA	PDA	PRA	BLE	BWI	Eigen-value	%
PCA 1								
1	0.784	0.856	0.738	0.670	0.796	0.353	3.098	51.64
2	0.050	-0.265	-0.375	-0.024	0.214	0.878	1.030	17.17
PCA 2								
1	0.764	0.804	0.893	0.732	0.499	-0.496	3.060	51.00
2	0.343	0.382	0.192	-0.409	-0.159	0.731	1.028	17.13
PCA 3								
1	0.822	0.865	0.764	0.686	0.725	-0.292	3.098	51.49
2	0.240	0.188	0.022	-0.436	0.251	0.886	1.131	18.85

Fig. 5.1. Frequency of different larval stages (4, 6, 8-arm stage) during development in each combination of parental and larval treatment. Parental treatments are high ration of kelp plus mussel flesh (KM) or low ration of kelp (KL); larval treatments are high (5000 cells ml^{-1}) or low ration (500 cells ml^{-1}) of *Dunaliella tertiolecta*. Data are means (+SE) of 3 jars (8-29 larvae per jar) per treatment combination.

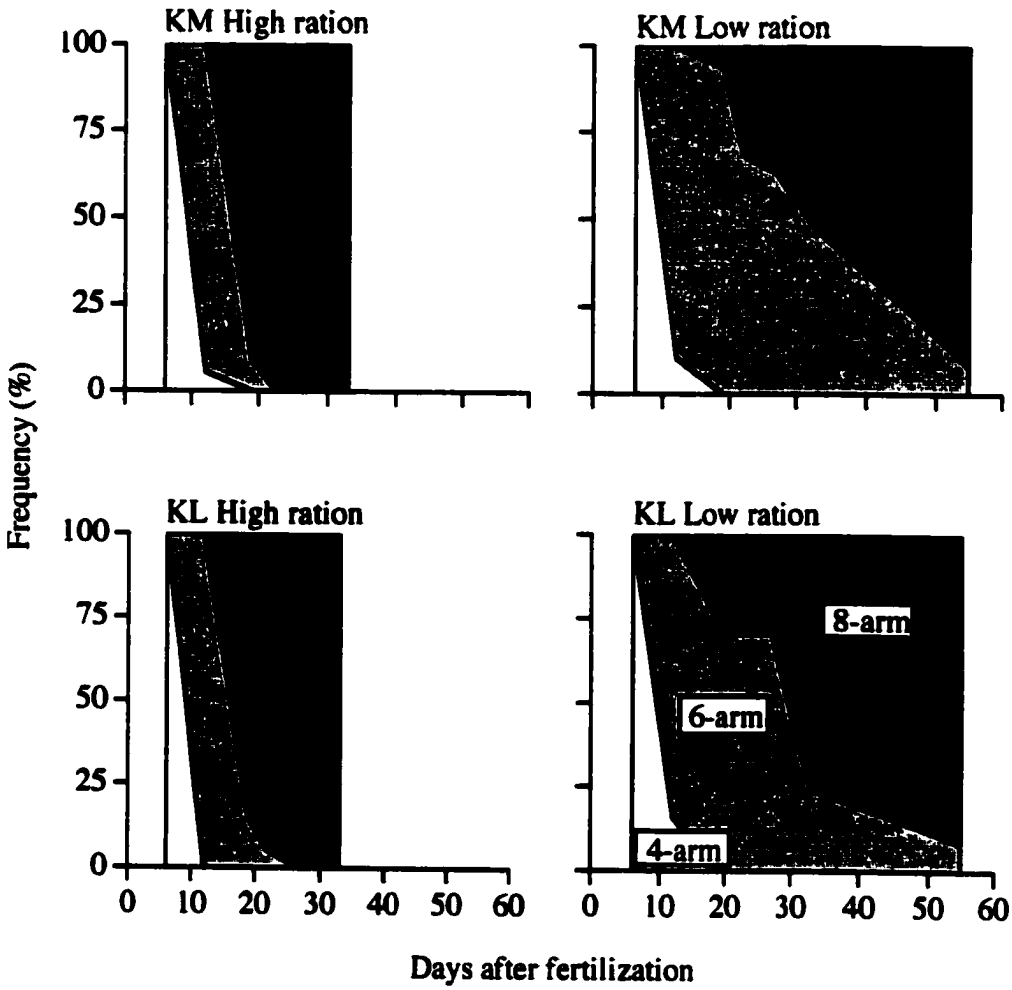
KM High ration
KM Low ration
KL High ration
KL Low ration
8-arm
6-arm
4-arm
Frequency (%)
100
75
50
25
0
0 10 20 30 40 50 60
Days after fertilization

Figure 5.1

Fig. 5.2. Changes in mean (+SE, often hidden behind symbols) length of larval arms (postoral, anterolateral, posterodorsal, and preoral), and body length and width, during development in each combination of parental and larval treatment (see Fig. 5.1 for abbreviations). Data are calculated from measurements of 6-10 larvae per jar for each of 3 jars pooled for each treatment combination.

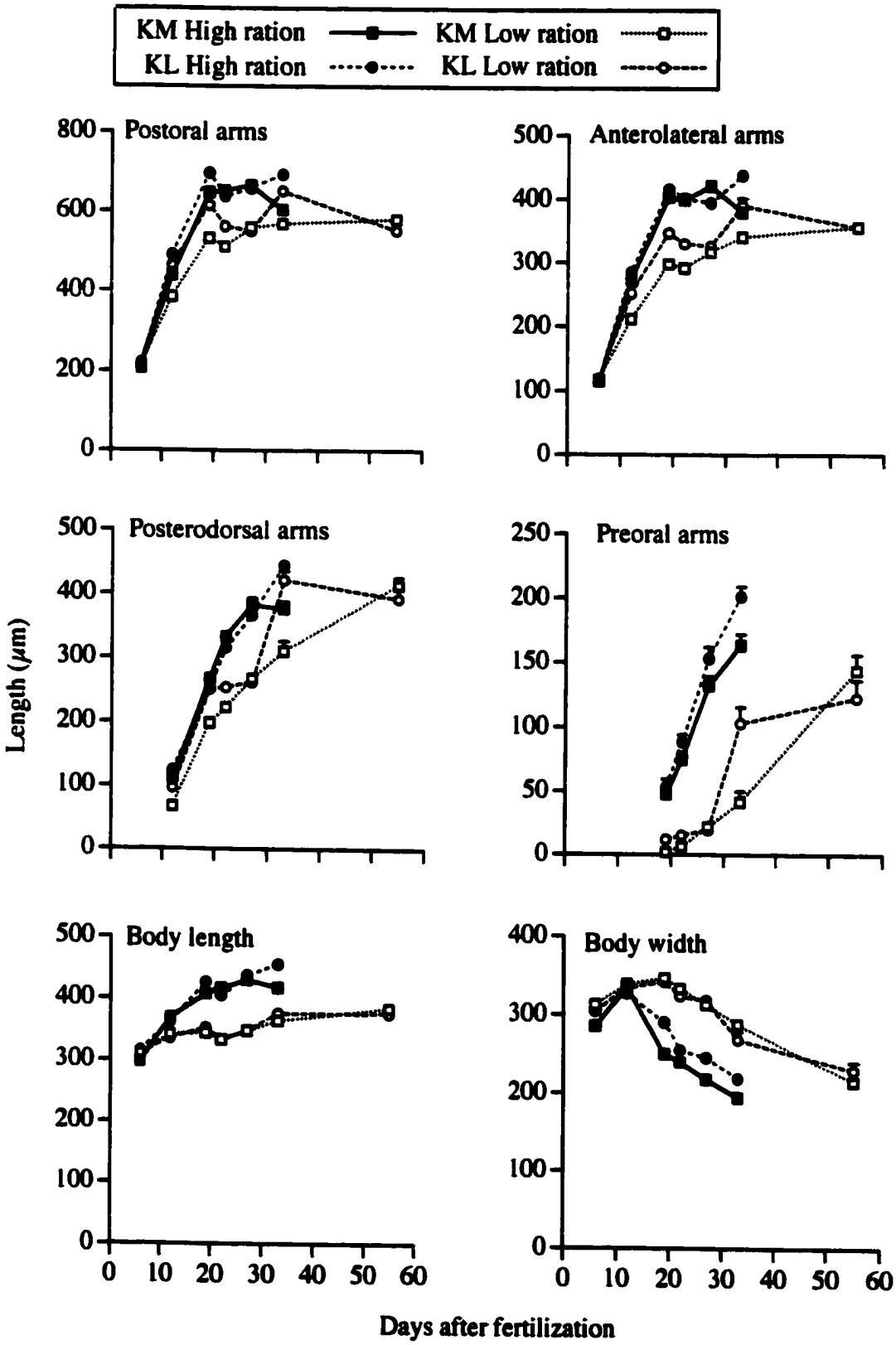
KM High ration
KM Low ration
KL High ration
KL Low ration
Postoral arms
Anterolateral arms
Posterodorsal arms
Preoral arms
Body length
Body width
Length (μm)
Days after fertilization

Figure 5.2

Fig. 5.3. Mean scores for the first and second principal component in a principal components analysis (PCA) based on measurements of larval arms and body size (see Fig. 5.2) at 33 and/or 55 d after fertilization from each combination of parental and larval treatment (see Fig. 5.1 for abbreviations). Means are calculated from scores of 10 larvae per jar (9 in one case) for each of 3 jars pooled for each treatment combination.

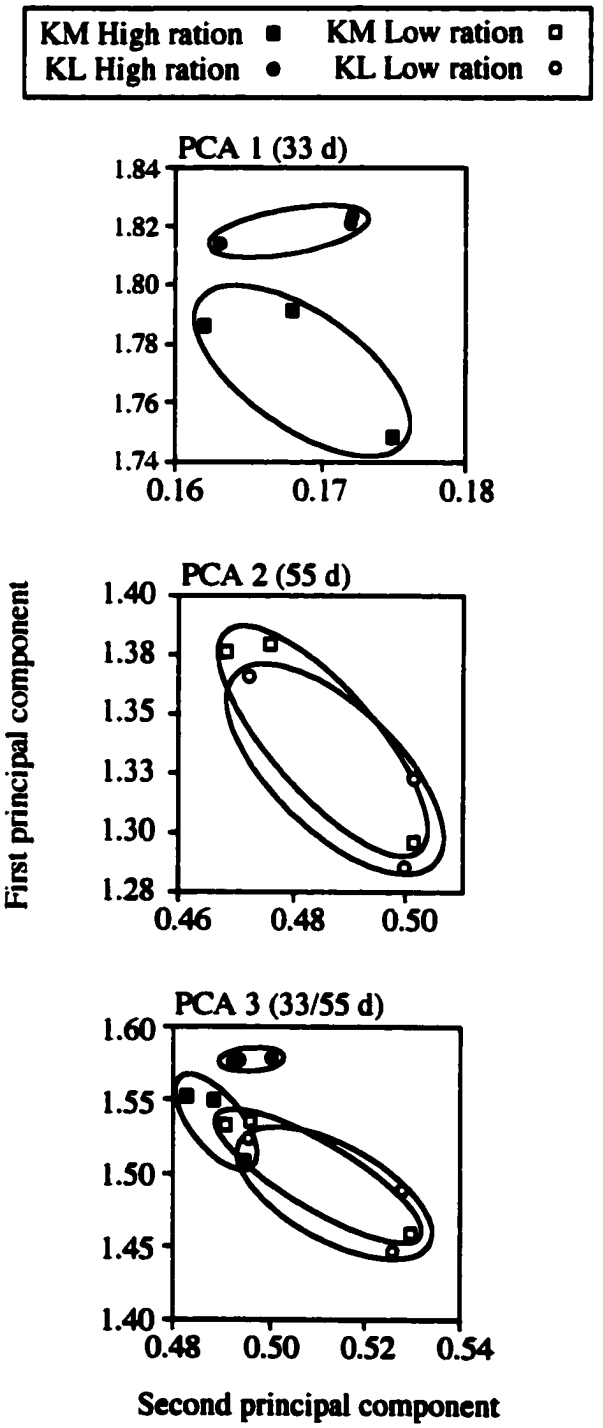
KM High ration
KM Low ration
KL High ration
KL Low ration
PCA 1 (33 d)
1.84
1.82
1.80
1.78
1.76
1.74
0.16
0.17
0.18
PCA 2 (55 d)
1.40
1.38
1.35
1.33
1.30
1.28
0.46
0.48
0.50
PCA 3 (33/55 d)
1.60
1.55
1.50
1.45
1.40
0.48
0.50
0.52
0.54
First principal component
Second principal component

Figure 5.3

Fig. 5.4. Changes in relative abundance of larvae (as a percentage of the initial number per jar at 2 d after fertilization) during larval development in each combination of parental and larval treatment (see Fig. 5.1 for abbreviations). Data are means (+SE) of three jars per treatment combination.

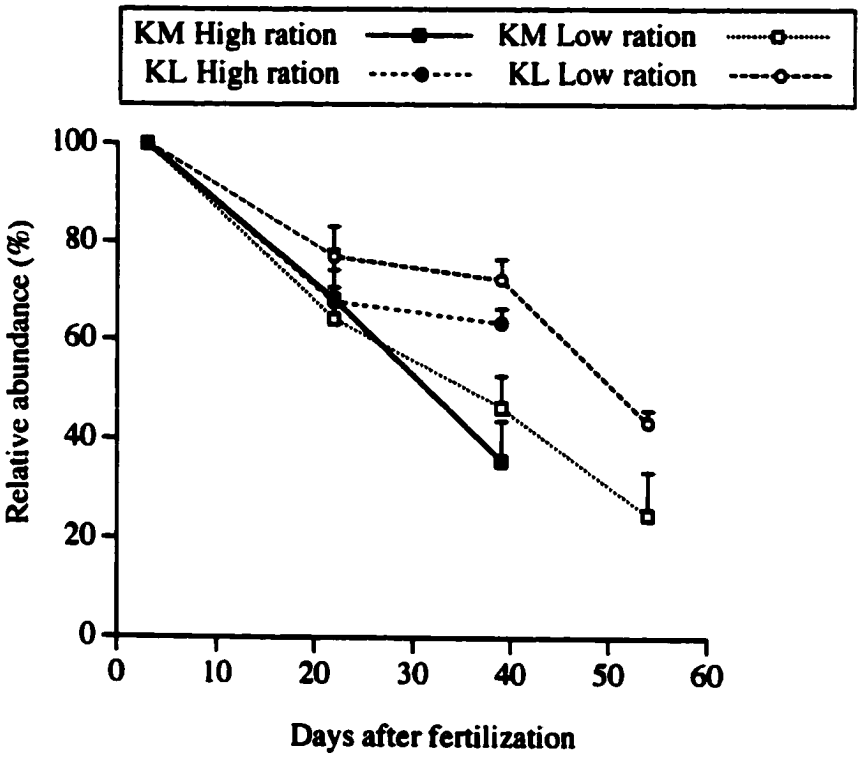
KM High ration
KM Low ration
KL High ration
KL Low ration
Relative abundance (%)
100
80
60
40
20
0
0
10
20
30
40
50
60
Days after fertilization

Figure 5.4

Fig. 5.5. Mean (+SE) rate of metamorphosis in each combination of parental and larval treatment (see Fig. 5.1 for abbreviations). Larvae fed the high ration were induced at 33 or 36 d after fertilization, those fed the low ration at 55 d. Sample sizes are given above bars.

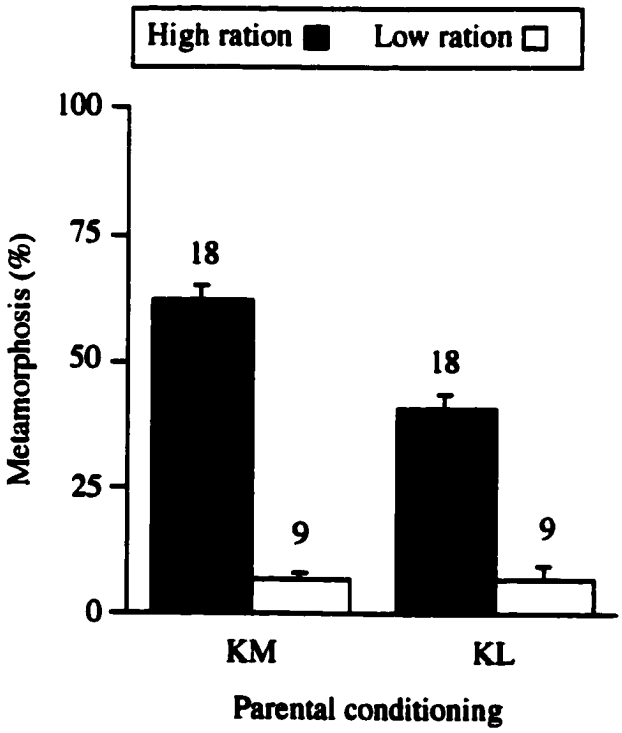
High ration
Low ration
100
75
50
25
0
Metamorphosis (%)
18
9
18
9
KM
KL
Parental conditioning

Figure 5.5

Fig. 5.6. Mean (+SE) test diameter of settlers in each combination of parental and larval treatment (see Fig. 5.1 for abbreviations) after 24 h and 1 wk. Sample sizes are given above bars.

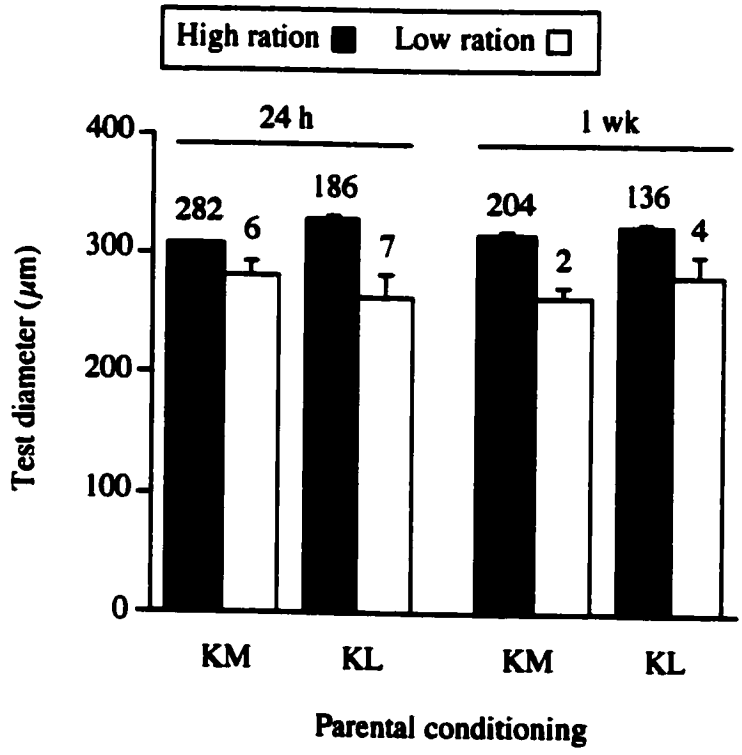
High ration
Low ration
24 h
1 wk
400
300
200
100
0
282
6
186
7
204
2
136
4
Test diameter (μm)
KM
KL
KM
KL
Parental conditioning

Figure 5.6

DISCUSSION

Larval nutrition had a much more pronounced effect than parental conditioning on the rate of larval development and growth in *Strongylocentrotus droebachiensis*. Larvae fed a high ration were larger throughout most of the developmental period and reached competence 22 d earlier than larvae fed the low ration. These results are consistent with previous studies of *S. droebachiensis* (Hart and Scheibling 1988a, b, Bertram and Strathmann 1998), other sea urchins (Paulay et al. 1985, Boidron-Métairon 1988, Strathmann et al. 1992, Hart and Strathmann 1994), and sea stars (Lucas 1982, Basch 1996, Basch and Pearse 1996). In contrast, the effects of parental conditioning amounted to relatively small differences in overall larval size or arm length in both larval rations on two sampling dates. These differences likely reflect a temporary divergence in growth or developmental rate between treatments. In previous studies with *S. droebachiensis*, the effect of parental conditioning on larval growth has been consistently small (de Jong-Westman et al. 1995b, Bertram and Strathmann 1998) although de Jong-Westman et al. (1995b) found that the effect varied in direction among several morphological characters. Small positive effects of parental conditioning on larval size and arm length also were detected in *Arbacia lixula* (George 1990).

There was a large positive effect of larval ration on the rate of metamorphosis (explaining 76% of the variance) as well as an effect on test diameter of recently metamorphosed juveniles in my study. Similar effects have been recorded for the sand dollar *Dendraster excentricus* (Hart and Strathmann 1994), and the starfish *Asterina miniata* (Basch and Pearse 1996), but were not observed in a previous study of *S. droebachiensis* (Hart and Scheibling 1988a). A small effect of parental conditioning on the rate of metamorphosis (2% of the variance) was evident only in the high ration, where significantly more larvae metamorphosed in the KM than the KL treatment. This

difference may reflect slightly accelerated development in the KM treatment as larvae reached competence. Parental conditioning had no effect on the size of recently metamorphosed juveniles. These results are consistent with the findings of de Jong-Westman et al. (1995b) for *S. droebachiensis* and George et al. (1990) for *Arbacia lixula*. Test diameter remained relatively constant in the first week after settlement, indicating that any effect of larval ration or parental conditioning on early post-metamorphic growth was negligible.

I quantified the decline in larval number in the culture jars as another measure of settlement rate. Fewer larvae remained in the high than the low ration at 39 d because of settlement in the high ration (which had started at ~33 d). In contrast, the small number of competent larvae and low rate of metamorphosis in the low ration at 55 d suggest that poor survival, and not settlement, accounted for the progressive decline in larval number in this treatment (see Hart and Scheibling 1988a for similar survival rates under similar conditions). Parental conditioning had a large effect on the decline in larval number (62% of the variance), but only in the high ration. Significantly fewer larvae remained in the KM than the KL treatment at 39 d, which was consistent with the greater rate of metamorphosis in the KM treatment in the induction trials.

My study showed that larval nutrition has a much more pronounced effect than parental conditioning on larval growth, development and rate of metamorphosis (see also Bertram and Strathmann 1998). This result is not surprising because planktotrophic larvae obtain most of their energy from the surrounding seawater through ingestion of particles such as phytoplankton and bacteria (reviewed by Boidron-Métairon 1995). A significant effect of parental conditioning on larval development implies a beneficial modification of the chemical composition of the egg that continues to exert its influence during the larval stage and perhaps into the early post-metamorphic stage. It has been

shown that the protein and lipid content of eggs, larvae and post-larvae is greater when the adult sea urchins are in better nutritional condition (George 1990, George et al. 1990, 1997). In my study, the greater rate of metamorphosis in the KM than the KL treatment may have been a consequence of some chemical component (e.g., protein or lipids) that was sequestered in greater concentrations in the eggs of females from the KM than the KL treatment, either because of the increased quantity of kelp or the inclusion of mussel flesh.

The observed positive effects of parental and larval nutritional condition on larval developmental and post-metamorphic traits may influence the fate of larvae and settlers in various ways. Rapid growth can be advantageous if increased larval size affords protection from planktonic predators (Rumrill and Chia 1984, Rumrill et al. 1985, Pennington et al. 1986). Because larval mortality due to predation, advection, or physiological stress is high (reviewed by Morgan 1995), an abbreviated larval phase may increase larval survival and hence settlement rate (Thorson 1950). Although little is known about the causes of settler mortality, micropredators may play an important role (Scheibling 1996). Increased size of settlers may reduce vulnerability to micropredators and hence rates of post-settlement mortality.

A rigorous examination of the relative effects of parental and larval nutritional condition on larval development in natural populations requires controlled conditioning during both life stages and the use of natural diets. Previous studies have used adults from different habitats, assuming that observed differences in gonad index reflect differences in egg quality (i.e., chemical composition) and ignoring the potentially confounding factor of geographic and/or genetic separation (Bertram and Strathmann 1998, George 1990, 1996, George et al. 1990), or have used artificial diets without any indication of how these relate to natural diets (de Jong-Westman et al. 1995a). In my

study, juveniles originated from a single locality and were reared to adulthood on natural food items (Himmelman and Steele 1971, Briscoe and Sebens 1988).

An assessment of the relative importance of parental and larval nutrition for larval growth and survival must consider natural variations in food supply for both larvae and adults. The low and high larval rations used in this study approximate chlorophyll *a* levels off the coast of Nova Scotia in mid-winter and during a 'small' spring phytoplankton bloom, respectively (Hart and Scheibling 1988a). As larvae of *S. droebachiensis* are present in the water column during the spring bloom in this region, they are probably not food-limited under natural conditions. The natural diet of adults of *S. droebachiensis* differs markedly among habitats (Keats et al. 1984, Chapters 2, 3), and most likely is intermediate to the KM and KH treatments in kelp beds and similar to the KL treatment in barrens (Minor and Scheibling 1997). The KM treatment may have been exceptional as it produced the highest gonad index reported for this species (Chapter 4). However, even this particularly good diet had little influence on most characteristics of larvae and settlers.

Although parental condition generally had a small effect on larval development of *Strongylocentrotus droebachiensis*, my results suggest that when planktonic food is abundant, larvae of adults from nutritionally rich habitats (such as kelp beds) may metamorphose sooner than those of adults from nutritionally poor habitats (such as barrens). The differential contribution to the overall pool of competent larvae, in terms of both number and quality, may result in differences in reproductive success between habitats, which in turn may influence the population dynamics of sea urchins in shallow water communities.

Chapter 6: Variation in zygote production and population dynamics of sea urchins

INTRODUCTION

In marine benthic invertebrates with a planktonic larval stage, variation in the supply of settling larvae to adult populations is an important determinant of population structure and dynamics (e.g., Pearse and Hines 1987, Minchinton and Scheibling 1991, Grosberg and Levitan 1992). The overall supply of settlers is determined by the total number of zygotes initially produced at a multitude of sites (Gaines and Lafferty 1995). Because of marked spatial and temporal variability in environmental (e.g., food supply, hydrodynamic regime) and demographic factors (e.g., population density, size structure, gamete output), different adult populations do not contribute equally to the total zygote pool. To date, there have been few attempts to evaluate how populations vary in their relative contribution to that pool (Keats et al. 1984), or how the size of the pool may vary over time. Empirical analysis of this problem is hampered by logistical difficulties. However, existing models of fertilization kinetics, in combination with our increased understanding of fertilization ecology (Levitan 1995), enable a theoretical approach.

Zygote production in free-spawning marine invertebrates is determined by the number of eggs spawned and the proportion of those eggs that is fertilized. Fecundity and fertilization rate are influenced in turn by the simple and interactive effects of a variety of factors. A high density of spawning adults generally increases the number of eggs spawned per unit area although it may negatively influence body size or gamete output of individual adults (e.g., Branham et al. 1971, Scheibling 1981, Levitan 1988a,

1991). Fertilization rate also tends to increase with adult density because sperm is less diluted when it encounters eggs if spawners are closely spaced (Pennington 1985, Denny and Shibata 1989, Levitan 1991, Grosberg 1991, Oliver and Babcock 1992, Levitan and Young 1995, Yund 1995), and because eggs may drift past several sperm sources (Levitan and Young 1995, Coma and Lasker 1997). Egg production will directly influence the number of zygotes produced, but the effect of sperm production on fertilization rate is more equivocal. Babcock et al. (1994) and Yund (1998) have reported that higher sperm output increases fertilization rate in the starfish *Acanthaster planci* and the ascidian *Botryllus schlosseri*, whereas Levitan (1991) did not observe such an effect in the sea urchin *Diadema antillarum*. Physical factors also influence fertilization rate. Empirical (Pennington 1985, Levitan et al. 1992, Wahle and Peckham in press) and theoretical (Denny and Shibata 1989, Young et al. 1992, Levitan and Young 1995) studies indicate that a low flow regime during spawning increases fertilization rate by reducing gamete dilution.

Well-studied species which exhibit large fluctuations in population density, demography, and reproductive output are ideally suited for modelling zygote production. One such species is the sea urchin *Strongylocentrotus droebachiensis* (Müller), for which changes in population size and structure have been well-documented along the Atlantic coast of Nova Scotia over the last three decades (Lang and Mann 1976, Wharton and Mann 1981, Scheibling et al. submitted). When sea urchins are in low abundance, kelp beds (consisting mainly of the kelps *Laminaria longicruris* and *L. digitata* with an understorey of coralline and fleshy macroalgae) thrive in the shallow rocky subtidal zone (Edelstein et al. 1969, Mann 1972a). Sea urchins exposed to an abundance of food in the form of drift algae and small understorey plants grow rapidly and produce large gonads (Lang and Mann 1976, Chapters 2, 3). As the density of sea

urchins increases, large individuals start to aggregate at the edge of kelp beds, forming fronts (Lang and Mann 1976, Wharton 1980b, Bernstein et al 1981, Scheibling et al. 1994, Scheibling et al. submitted) which destructively graze the kelp. Sea urchins in fronts climb up onto plants, weigh them down and consume them entirely (Breen and Mann 1976a, Mann 1977). The facilitative effect of group feeding on an abundant food source, attached kelps, enables sea urchins in this zone to grow and reproduce at a high rate despite high densities (Chapters 2, 3). The formation of grazing fronts initiates a large-scale transition in community state as fronts advance into the kelp bed and create, in their wake, barren grounds dominated by coralline algae (Bernstein et al. 1981, Johnson and Mann 1986, Chapman and Johnson 1990). This transition from the kelp bed state to the barrens state occurs within about a decade in Nova Scotia (Breen and Mann 1976a, Scheibling et al. submitted). After the destruction of kelp beds, sea urchins in barrens remain relatively abundant but their nutritional state deteriorates, leading to declines in growth and reproductive rates (Lang and Mann 1976, Chapters 2, 3). The subtidal zone persists in the barrens state until sea urchins are eliminated by disease (Miller 1985a, Scheibling 1986). The ensuing reduction in grazing pressure results in rapid growth of macroalgae, and kelp beds are reestablished within 2-3 years (Scheibling 1986, Novaczek and McLachlan 1986, Johnson and Mann 1988). Following mass mortalities, sea urchins begin to recolonize the developing kelp beds via larval settlement (Miller 1985a, Raymond and Scheibling 1987), but the recovery of adult populations may be limited by intensive predation on recruits (Scheibling 1996). The mechanisms causing population outbreaks of *S. droebachiensis* have not yet been conclusively demonstrated, but increased survival of the planktonic larvae (Hart and Scheibling 1988a) and migration of sea urchins from deeper waters into the shallows

(Scheibling et al. submitted) have been hypothesized. The role of zygote production by sea urchins in initiating outbreaks has not been explored.

In this paper, I construct a model of fertilization kinetics to predict zygote production per unit area, and on a coastal scale, by *S. droebachiensis* in kelp beds, barrens, and grazing fronts along the Atlantic coast of Nova Scotia. I parameterize this model using sea urchin population data and hydrodynamic measurements obtained from my own work (Chapters 2-4), the literature and unpublished observations. I then estimate the relative contributions of sea urchins in these different habitats to the total pool of zygotes produced during the transition from kelp beds to barrens. My goal is to evaluate the importance of spatial and temporal variation in zygote production to the population dynamics of *S. droebachiensis* along this coast. In addition, my modelling approach enables me to identify gaps in our knowledge which will guide future studies on the dynamics of the ecosystem as a whole.

MODEL CONSTRUCTION AND PARAMETER ESTIMATION

Density and demography of subpopulations

Fluctuations in populations of *Strongylocentrotus droebachiensis*, and hence in community state, occur along much of the Atlantic coast of Nova Scotia where disease outbreaks periodically eliminate sea urchins (Miller 1985a, Scheibling 1986, Scheibling and Hennigar 1997; Fig. 6.1). In my model I include three different community states and sea urchin subpopulations in this region of coast: 1) the established kelp bed state with sea urchins in kelp beds; 2) the transition state with sea urchins in kelp beds, transitional barrens, and grazing fronts; and 3) the barrens state with sea urchins in post-transitional barrens. I also consider the other transition state when kelp beds develop following a mass mortality of sea urchins. Although sea urchins recruit to the

developing kelp beds, this state is distinguished from the established kelp bed state by the absence of adult sea urchins and therefore not included in my model.

To parameterize my model, I compile data on the population density, the proportion of adults, and the mean and maximum body size of adults in each of the three subpopulations I consider (Table 6.1). These data come from investigations on populations of *S. droebachiensis* along the Atlantic coast of Nova Scotia over a 25-year period. I estimate the proportion of adults, and the mean and maximum adult size from recorded size frequency distributions which I reconstruct (where necessary) in 5 mm size classes. The proportion of adults depends on the size (test diameter) at first reproduction which ranges between 18 and 25 mm in *S. droebachiensis* (Vadas 1977, Thompson 1979, Scheibling 1986, Raymond and Scheibling 1987, Munk 1992). Because sea urchins tend to reproduce earlier in life, and therefore at a smaller size, in a nutritionally rich environment (Buchanan 1966, Sivertsen and Hopkins 1995, Chapter 4), I use 20 mm as the size at first reproduction in kelp beds and grazing fronts, and 25 mm in transitional and post-transitional barrens. Mean adult size is estimated by multiplying the mid-point of each 5 mm size class by the proportion of adult sea urchins in that class and summing the resulting values over all adult size classes.

Fertilization rate

Sperm concentration. I use the following model derived from Denny (1988, eq. 10.36) to calculate the concentration of sperm S (no. m^{-3}) at point x located at distance i (m) directly downstream from a spawning male of size j (weighted mean adult test diameter, mm) in subpopulation k (sea urchins in kelp beds, grazing fronts, transitional or post-transitional barrens):

$$S(x)_{i,j'_k,k} = \frac{Q_{j'_k}\,\bar{u}_k}{2\pi\left(\alpha(x)_y\right)_{i,k}\left(\alpha(x)_z\right)_{i,k}\left(u_*\right)_k^2\,x_{ik}^2} \qquad [6.1]$$

where Q is weighted sperm release rate (no. s^{-1}), $\bar{u}$ is mean current velocity (m s^{-1}) in the x direction, u_* is friction velocity (m s^{-1}), and α_y and α_z are the diffusion coefficients of the sperm plume dispersing along the horizontal and vertical axes perpendicular to x, respectively. This model describes gamete dispersion within the turbulent benthic boundary layer (Denny 1988). All symbols used in this model and in subsequent equations are given on pages xvii-xix.

Weighted mean adult size j' in subpopulation k (Table 6.2) is estimated from published data (Table 6.1) and calculated as:

$$j'_k = \sum_j \left(j\,p(j)_k\right) \qquad [6.2]$$

where j is the mid-point of a 5 mm size class of adults and p is the proportion of sea urchins in that size class of adults. The range of size classes of adults differs among subpopulations depending on size at first reproduction and the maximum size. For each subpopulation, the mean proportion of sea urchins in each size class of adults (Table 6.2) is estimated from the sources in Table 6.1 by summing the proportions in each class over all studies and dividing that sum by the total proportion in all adult classes. Because the proportion of sea urchins >60 mm is low (≤0.02) in all subpopulations, I use 55-60 mm as the largest size class.

Sperm concentration is estimated at multiple points x_i directly downstream from a spawning male. In each subpopulation k, the first point x_i is determined by the distance between the male and the nearest downstream spawning female, $r^{(1)}$. This distance is estimated using a nearest-neighbour method (Clark and Evans 1954), which assumes a random dispersion:

$$r_k^{(1)} = \frac{0.5}{\sqrt{\rho_k\, a_k\, b\, c_k^{(1)}\, f}} \qquad [6.3]$$

where ρ is sea urchin density (no. m^{-2}), a is the proportion of adults, b is the proportion of spawning adults, $c^{(1)}$ is the proportion of sea urchins downstream of the male, and f is the proportion of females. Thereafter, sperm concentrations are calculated at successive 0.05 m intervals. For convenience, $r_k^{(1)}$ is rounded to the nearest 0.05 m to coincide with those intervals (Table 6.2).

Mean density and mean proportion of adults in each subpopulation (Table 6.2) are estimated from published data (Table 6.1). The proportion of adults that spawn during one spawning event (Table 6.2) is estimated as the mean proportion of adult sea urchins that were partially spawned or spent on a single sampling date during the peak of the main spawning season (March to May), pooled over kelp beds, grazing fronts and transitional barrens (Chapter 2). Because *S. droebachiensis* usually has a sex ratio of 1:1 (Munk 1992, Chapter 2), I set the proportion of females to 0.5 in all subpopulations (Table 6.2).

The proportion of sea urchins downstream of a spawning male in subpopulation k (Table 6.2) is estimated by approximating the planar projection of the dispersing sperm plume as an imaginary triangle of height $x_{i,k}$ and base $((\alpha(x)_y)_{i,k})$ $(x_{i,k})$. The angle formed by the expanding plume (i.e., that which subtends the base of the imaginary triangle) is then calculated trigonometrically and expressed as a proportion of 360°:

$$c_k^{(1)} = \frac{1}{360}\left\{2\left[\arctan\left(\frac{\left(\alpha(x)_y\right)_{i,k}\, x_{i,k}}{2\, x_{i,k}}\right)\right]\right\} \qquad [6.4]$$

Distance x_i in equation [6.4] is calculated by iteration. I estimate a reasonable value of x_i and calculate $c^{(1)}$, which I then substitute into equation [6.3] and repeat this process until $x_{i,k}=r_k^{(1)}$.

The weighted sperm release rate Q (s^{-1}) at size j in subpopulation k (Table 6.2) is determined as

$$Q_{j,k} = \sum_j \left(\frac{G_j}{3.66 \times 10^{-12}}\right)\left(\frac{1}{60\,\delta_j}\right) p(j)_k \qquad [6.5]$$

where G is the dry weight (g) of sperm released, 3.66 x 10^{-12} is the dry weight (g) per sperm of *S. droebachiensis* (Thompson 1979), and δ is the spawning time (min) of a male sea urchin for size classes j=22.5-57.5 mm in kelp beds and grazing fronts, and j=27.5-57.5 mm in transitional and post-transitional barrens. Dry weight of sperm released at size j in subpopulation k is estimated from the spawned wet weight of gonads, and a wet to dry weight conversion coefficient for gonads of *S. droebachiensis* estimated by regression (r^2=0.970, n=87)

$$G_{j,k} = 0.249\left(I_{j,k}\, C_k\, W_j\right) \qquad [6.6]$$

where I is the maximum proportion of the total body wet weight that is gonads, C is the proportion of gonads that is spawned, and W (g) is the total wet body weight. In kelp beds, grazing fronts and transitional barrens, I estimate I (Table 6.2) using information presented in Chapter 2. Because I increases with size in young adult sea urchins, I estimate I for j=22.5-32.5 mm using a logistic equation (Chapter 2). For larger adults, I is at an asymptotic level and is estimated as the average I at the peak of the reproductive cycle, based on measurements over two years and at two sites for each subpopulation (Chapter 2). I assume that in post-transitional barrens I is 30% of that in kelp beds (Lang and Mann 1976). In kelp beds, grazing fronts and transitional barrens, I estimate

C, the proportion of gonads spawned (Table 6.2), based on my measures of the decrease in wet gonad mass directly after spawning (Chapter 2). I assume that sea urchins in post-transitional barrens release the same proportion of gonadal material as those in transitional barrens. I estimate W, the total wet body weight, at each size j using the following regression (r^2=0.995, n=424; Scheibling et al. submitted):

$$\ln W_j = 2.81 \ln j - 7.00 \qquad [6.7]$$

I measured spawning times for *S. droebachiensis* in the laboratory following induction with 0.53M KCl (unpubl. data). Because spawning time varies with gonad mass and body size, I estimate spawning time δ (min) of a sea urchin of size j in subpopulation k as

$$\delta_{j,k} = 110\left(\frac{I_{j,k}}{0.193}\right)\left(\frac{j}{34.1}\right) \qquad [6.8]$$

where 110 (min) is the mean spawning time (SD=19.3 min), 0.193 is the mean proportion of the total body weight that is gonads (SD=0.036), and 34.1 (mm) is the mean test diameter (SD=4.5 mm) of males spawned in the laboratory (n=9).

I used the following logarithmic equation (Denny 1988, eq. 9.24) to estimate $\bar{u}$ at a given height s (m) above the substratum in barrens:

$$\bar{u}(s) = \frac{u_*}{\kappa} \ln\left(\frac{s - d_0}{s_0}\right) \qquad [6.9]$$

where κ is von Karman's constant (0.41), s_0 is the roughness height, and d_0 is the zero plane displacement. Roughness height s_0 is the height at which $\bar{u}$ would be 0 if the logarithmic profile of the benthic boundary layer applied to heights <H, where H (m) is the height of roughness elements. Following Denny (1988), I assume that s_0=0.033 x H. I set H=0.08 m to account for surface irregularities in the rocky substratum. The zero plane displacement d_0 is the height to which the effective substratum has been raised as a

result of the presence of roughness elements (Denny 1988). I set d_0=0.6 x H, which is appropriate for many rough surfaces (Denny 1988). The friction velocity u_* is a function of the Reynold's shear stress and measures turbulent fluctuations in current velocity near the substratum (Csanady 1973, Denny 1988).

Mean current velocities in subpopulations were estimated from measurements collected in barrens at a sheltered site in Nova Scotia with low current flow (Balch and Scheibling unpubl. data) and at a site in Maine with strong tidal flow (Wahle and Peckham in press). For the low flow site, I estimate u_* from Equation [6.9] using a mean current velocity of 0.033 m s^{-1} (SD=0.005, n=3) measured by current meters (S4, Inter Ocean Systems, San Diego, California) in barrens at s=0.7 m over periods of 13-35 days. I then substitute u_* into equation [6.9] to calculate $\bar{u}$ at s=0.1 m, the height at which I estimate fertilization rate. At the high flow site, current velocity was measured using neutrally buoyant particles at s=0.1 m (mean velocity ±SD: 0.093 ±0.004 m s^{-1}, n=40). I average the values at the two sites to estimate the mean current velocity at s=0.1 m in transitional and post-transitional barrens and at grazing fronts (Table 6.2) and substitute this average velocity into equation [6.9] to calculate an average u_* at that height (Table 6.2).

Macroalgae are known to reduce current velocity (Jackson 1986, Eckman et al. 1989), and consequently turbulence and friction velocity (Eckman 1983). Wahle and Peckham (in press) found that current velocity in an experimental patch of *Laminaria saccharina* was ~25% of that in adjacent kelp-free areas at a height of 0.1 m. Therefore, I set $\bar{u}$ in kelp beds to 25% of $\bar{u}$ in barrens (Table 6.2). Previous fertilization studies employed values for u_* of 10% of $\bar{u}$ in the surf zone (Denny and Shibata 1989), in coral reefs (Babcock et al. 1994), and on flat rock pavement (Levitan and Young 1995), while Young et al. (1992) measured u_* to be 8% of $\bar{u}$ at s=2.3 m in the deep sea. My estimate

of u_* in barrens is 13.76% of $\bar{u}$ at s=0.1 m (Table 6.2). Staying within the general range employed by previous studies (i.e., around 10%), I reduce friction velocity in kelp beds by 2% relative to barrens to 11.76% of $\bar{u}$ (Table 6.2) to account for the influence of macroalgae.

I estimate the vertical gamete diffusion coefficient α_z at point x_i in subpopulation k as

$$\left(\alpha(x)_z^2\right)_{i,k} = \frac{\left(K_z\right)_k}{\left(u_*^2\right)_k \, l_k} \qquad [6.10]$$

where the vertical eddy diffusivity K_z in subpopulation k is estimated as

$$\left(K_z\right)_k = \kappa \left(u_*\right)_k s \qquad [6.11]$$

and l is the time (s) sperm take to advect to a point x_i (Denny 1988, eqs. 10.15, 10.18). To estimate $\alpha(x)_y$, I use the relationship $\alpha_y/\alpha_z = 1.6$ (Denny and Shibata 1989, Young et al. 1992).

Proportion of eggs fertilized. I use a fertilization kinetics model derived from Vogel et al. (1982, eq. 6) to calculate the proportion of eggs fertilized ($\varphi^{(P)}$) at point x_i downstream from a spawning male of body size j' in subpopulation k:

$$\varphi(x)^{(P)}_{i,j'_k,k} = 1 - e^{-\beta_k S(x)_{i,j'_k,k} \tau} \qquad [6.12]$$

where β (mm^3 s^{-1}) is the rate constant of fertilization, S is sperm concentration (no. μl^{-1}) at point x_i, and τ is sperm half-life (s). Hereafter, I use the terms 'proportion of eggs fertilized' and 'fertilization rate' (the proportion of eggs fertilized expressed as a percentage) interchangeably.

The rate constant of fertilization β is calculated as

$$\beta = \upsilon \sigma \qquad [6.13]$$

where υ is sperm swimming speed and σ is the fertilizable area of an egg (Vogel et al. 1982, eq. 7). This area is 16.6% of the egg cross-sectional area σ_0 (Levitan 1993), which for an egg of *Strongylocentrotus droebachiensis*, with a diameter of 0.145 mm (Levitan 1993), is 1.7×10^{-2} mm^2 (i.e., $\sigma = 2.8 \times 10^{-3}$ mm^2). Because sperm are more likely to be transported by the turbulent water masses into which they are spawned than by their own weak swimming powers (υ=0.088 mm s^{-1}, Levitan 1993), I follow the approach of Denny (1988) and Babcock et al. (1994) and replace sperm swimming speed with the friction velocity u_* (mm s^{-1}) in subpopulation k to give (Table 6.2)

$$\beta_k = (u_*)_k \sigma \qquad [6.14]$$

Sperm half-life τ depends on sperm concentration (Chia and Bickell 1983) and for *S. droebachiensis* can be as long as 3 h (Levitan 1993). Sperm half-life can be replaced by sperm-egg contact time t (s), the time an egg spends at a sperm concentration, when $t<\tau$ (Vogel et al. 1982). In my application of the fertilization model, sperm-egg contact time is the time required for gametes to travel 0.05 m, which depends on the mean current velocity $\bar{u}$. Because sperm-egg contact time is brief at the velocities I estimated (Table 6.2), I replace τ by t.

Equation [6.12] estimates the proportion of the eggs of a female at point x_i that is fertilized by the nearest upstream male. As sperm and eggs are travelling downstream together, further fertilizations can occur. Also, in the presence of multiple upstream spawning males, the eggs of one female can be fertilized by more than one male. I estimate the cumulative proportion ($\varphi^{(C)}$) of eggs that is fertilized by multiple males of size j' in subpopulation k using the following model derived from Denny (1988, eq. 10.40):

$$\varphi_{j_k',k}^{(C)} = \left(\varphi(r_k^{(1)})_{j_k',k}^{(M)}\right) + \left(\varphi(r_k^{(1)}+0.05)_{j_k',k}^{(M)}\right)\left[1-\left(\varphi(r_k^{(1)})_{j_k',k}^{(M)}\right)\right] + \left(\varphi(r_k^{(1)}+0.10)_{j_k',k}^{(M)}\right)\left\{1-\left(\varphi(r_k^{(1)}+0.05)_{j_k',k}^{(M)}\right)\left[1-\left(\varphi(r_k^{(1)})_{j_k',k}^{(M)}\right)\right]\right\}.. \quad [6.15]$$

where $\left(\varphi(r_k^{(1)})_{j_k',k}^{(M)}\right)$ is the proportion of eggs fertilized by multiple males of size j' in subpopulation k which is estimated as

$$\varphi(x)_{i,j_k',k}^{(M)} = 1 - e^{-\beta_k \sum_{n=1}^{m}\left[\left(S(x)_{i,j_k',k}\right)_n\right]_t} \quad [6.16]$$

at $x_i = r_k^{(1)}$. Similarly, $\left(\varphi(r_k^{(1)}+0.05)_{j_k',k}^{(M)}\right)$ is estimated as $\varphi(x)_{i,j_k',k}^{(M)}$ at one 0.05 m interval beyond $r_k^{(1)}$, and so on. The term m denotes the total number of males that contribute sperm to the plume and the term n the nth nearest spawning male upstream from the female. Equation [6.15] accounts for the decrease in the number of virgin eggs due to successful fertilizations (Denny 1988) and equation [6.16] estimates the proportion of eggs fertilized by the sperm of multiple males at point x_i. In equation [6.15], I sum the proportion of eggs fertilized over several points x_i which move downstream in 0.05 m increments from $r_k^{(1)}$. To calculate the distance from the female to the 2nd ($r_k^{(2)}$), 3rd ($r_k^{(3)}$), or 4th ($r_k^{(4)}$) nearest upstream spawning male (Table 6.2), I use Thompson's (1956) extension of the nearest-neighbour method of Clark and Evans (1954) and replace 0.5 in the numerator of equation [6.3] with 0.75, 0.9375, or 1.0937, respectively. I calculate the proportion of female sea urchins downstream of the 2nd ($c_k^{(2)}$), 3rd ($c_k^{(3)}$), or 4th ($c_k^{(4)}$) spawning male using equation [6.4]. Summations in equation [6.15] are terminated where $\varphi(x)_{i,j_k',k}^{(M)}<5\%$. I set this cut-off to offset my overestimation of fertilization rate within the plume by not accounting for a decrease in sperm concentration from the centre towards the sides of the plume (Denny 1988,

Denny and Shibata 1989). Males are added to summations until the addition of the *n*th male increases $\varphi^{(C)}_{j_k,k}$ by <5%. To assess sperm viability at the point where summations are terminated, I use a regression of sperm half-life on sperm concentration in *S. droebachiensis* (Levitan 1993).

Eggs spawned per unit area

I calculate the total number of eggs spawned (O) per unit area (U, m^2) in subpopulation k as the sum of the eggs spawned by females in all size classes of adults:

$$O_k^{(U)} = \sum_j \left(\rho_k \, a_k \, p(j)_k \, f \, \frac{F_j}{5.56 \times 10^{-7}} \right) \qquad [6.17]$$

where F is fecundity (dry weight of eggs released, g) of a female sea urchin and 5.56 x 10^{-7} is the dry weight per egg (g, Thompson 1979). Fecundity is calculated in the same way as G, the dry weight of sperm released by a male sea urchin, using equations [6.6] and [6.7] for size classes j=22.5-57.5 mm in kelp beds and grazing fronts, and j=27.5-57.5 mm in transitional and post-transitional barrens.

Zygotes produced per unit area

The number of zygotes produced ($Z^{(U)}$) per unit area U in subpopulation k is calculated as

$$Z_k^{(U)} = O_k^{(U)} \varphi^{(C)}_{j_k,k} \qquad [6.18]$$

Zygotes produced on a coastal scale

The number of zygotes produced (Z) on a coastal scale L in subpopulation k is calculated as:

$$Z_k^{(L)} = Z_t^{(U)} A_k \qquad [6.19]$$

where A is the total area occupied by a subpopulation (km^2).

The total number of zygotes produced by subpopulations combined in the shallow rocky subtidal zone T is calculated as

$$Z^{(T)} = \sum_k Z_k^{(L)} \qquad [6.20]$$

I consider only subpopulations along that section of the coast of Nova Scotia where near-complete mass mortalities of *Strongylocentrotus droebachiensis* have been documented (Fig. 6.1). I also limit the offshore distribution of these subpopulations to a depth of 15 m, where >90% of the total algal biomass in this region is found (Mann 1972a, Moore and Miller 1983). The total area occupied by a subpopulation is estimated using surveys of the shallow (to 15 m depth) subtidal zone of the Atlantic coast of Nova Scotia by Moore and Miller (1983) and Moore et al. (1986). These surveys determined the total area (km^2) that consisted of >50% hard substratum and was deemed suitable habitat for sea urchins and macroalgae. The section of the coast I consider includes the entire area surveyed by Moore and Miller (1983), which they estimate to have hard substratum covering 512 km^2. Of the area surveyed by Moore et al. (1986), only the portion of eastern Halifax county (~27 km^2) is relevant here, which brings the total estimated area of suitable habitat for sea urchins and macroalgae to 539 km^2.

When the shallow subtidal zone is in the established kelp bed or the barrens state, the entire area is occupied by sea urchins either in kelp beds or in post-transitional barrens. During the transition state, when kelp beds are progressively replaced by transitional barrens, I incrementally decrease the area of kelp beds by 1% over time, and correspondingly increase the area of barrens. Because transitional barrens gradually change into post-transitional barrens, I decrease (increase) the area of transitional (post-

transitional) barrens incrementally by 10% from the onset of the barrens state until the entire rocky subtidal zone is converted to post-transitional barrens.

The total area represented by grazing fronts on a coastal scale is estimated by assuming a front width of 2 m (Bernstein et al. 1981, Scheibling et al. submitted) along the entire length of the rocky coast. As grazing fronts may be convoluted or discontinuous along the edge of a kelp bed (pers. obs.), my estimate of the area represented by grazing fronts is a rough approximation. The surveys (Moore and Miller 1983, Moore et al. 1986) give a total length of coastline suitable for sea urchins of 1609 km (1400 km for the southwestern shore and 209 km for eastern Halifax County). Based on these estimates of front width and length, the total area occupied by grazing fronts is ~3.2 km^2. This area is assumed to be constant during most of the transition state, and subtracted from the estimated area of transitional barrens. Because grazing fronts form and disperse over time, I incrementally increase the area occupied by this subpopulation in the early stages of the transition state by 10% and similarly decrease it in the early stages of the barrens state.

MODEL PREDICTIONS

Fertilization rate

Sperm concentration (Fig. 6.2) and the proportion of eggs fertilized at a given distance downstream from a single spawning male (eq. [6.12]; Fig. 6.3a) show a similar pattern in all subpopulations: both are very high within a few cm of the male, drop off rapidly within the first meter, and then gradually decrease to very low values at greater distances. Sperm concentration and fertilization curves are almost identical in grazing fronts and barrens, while in kelp beds, where currents are weaker, sperm

concentration and fertilization rate decrease more slowly with distance and remain at an elevated level for longer.

When fertilizations are effected by multiple males (eq. [6.16]), the proportion of eggs fertilized at a given distance downstream (from the nearest spawning male) declines more slowly in all four subpopulations (Fig. 6.3b) than if only the nearest male is spawning (Fig. 6.3a). Once the sperm plume has reached the downstream spawning female and virgin eggs are removed from the gamete cloud (eq. [6.15]), fertilization rate drops sharply and rapidly approaches zero (Fig. 6.3b). Under these conditions, my calculations show that fertilization rate is highest in grazing fronts where two males fertilize 97% of the eggs of the female (0.25 m from the nearest male) within 0.15 m downstream of that female, or within 7 s after the males start to spawn. Fertilization rates in kelp beds and post-transitional barrens are similar at 93% for the same number (four) of simultaneously spawning males and within the same distance downstream of the spawning female (0.2 m). However, this rate is attained for females at different downstream distances from the nearest spawning male in each habitat (1.90 and 0.65 m in kelp beds and post-transitional barrens, respectively) and within different times after the males start to spawn (150 and 15 s, respectively). Fertilization rate is lowest in transitional barrens where three males fertilize 89% of the eggs of the downstream female (0.75 m from the nearest male) within 0.25 m downstream of that female, or within 18 s after the males start to spawn.

Eggs spawned and zygotes produced

I estimate that the total number of eggs spawned per m^2 differs among subpopulations by more than one order of magnitude (Fig. 6.4). It is highest in grazing fronts (7.1×10^7), intermediate in transitional and post-transitional barrens (5.8×10^6

and 4.4 x 10^6, respectively), and lowest in kelp beds (1.0 x 10^6). Because predicted fertilization rates are very high in all subpopulations, the pattern of zygote production per unit area mirrors that of the numbers of eggs spawned (Fig. 6.4).

The temporal variation in the number of zygotes produced by the different subpopulations on a coastal scale (Fig. 6.5) is related to changes in the extent of the subtidal zone that these subpopulations occupy. Zygote production in kelp beds is highest when the system is in the established kelp bed state (5.2 x 10^{14}) and declines at an accelerating rate as this habitat is destroyed during the transition state. In the expanding transitional barrens, zygote production concomitantly increases to reach a maximum (2.8 x 10^{15}) late in the transition state once kelp beds have been destroyed. While grazing fronts are fully established, sea urchins in this zone occupy, according to my assumption, a constant area and produce a constant supply of zygotes (2.2 x 10^{14}). In the post-transitional barrens after the fronts have dispersed, zygote production (2.2 x 10^{15}) is somewhat lower than in transitional barrens but over four-fold higher than in kelp beds in the established kelp bed state.

I predict total zygote production by *Strongylocentrotus droebachiensis* in the shallow subtidal zone to vary markedly in time depending on the community state (Fig. 6.5). Zygote production is lowest in the established kelp bed state and progressively increases during the transition state as kelp beds are replaced by transitional barrens, reaching a maximum (3.0 x 10^{15}) just before grazing fronts disperse. Although sea urchins in fronts occupy <1% of the total subtidal area, they contribute 4-7% of all zygotes produced during the transition state. As the entire subtidal zone is converted to post-transitional barrens, total zygote production drops by ~22% (to 2.2 x 10^{15}) in the barrens state.

SENSITIVITY ANALYSIS

I analysed mean proportional sensitivity (E, also termed elasticity) based on all four subpopulations to measure how sensitive a model output is to small changes in parameters:

$$E = \frac{1}{4}\sum_{k}\left(\frac{\frac{X_k^* - X_k}{X_k}}{\frac{v_k^* - v_k}{v_k}}\right) \qquad [6.21]$$

where X^* is the output of the altered model, X is the output of the original model, v^* is the parameter value increased by a small amount, and v is the original parameter value. A proportional sensitivity of 1 means that the model output increases by the same proportion by which the parameter value is increased. Sensitivity analysis indicates which parameters need to be most carefully estimated to obtain accurate model predictions. For my purposes, the analysis also indicates which parameters may be important for explaining differences in model output among subpopulations.

I calculated the effect of an increase in a basic parameter by 1% on the cumulative proportion of eggs fertilized by multiple males ($\varphi^{(C)}_{j_i,k}$, eq. [6.15]) and on the number of eggs spawned per unit area ($O^{(U)}_k$, eq. [6.17]). Basic parameters for $\varphi^{(C)}_{j_i,k}$ were mean current velocity, friction velocity, and the proportions of body weight that is gonads in males, gonads that is spawned by males, and males in a size class. Basic parameters for $O^{(U)}_k$ were population density, and the proportions of adults, females, body weight that is gonads in females, gonads that is spawned by females, and females in a size class. Only one parameter was varied in each analysis with the exception of mean current velocity and friction velocity for which derived parameters were varied simultaneously. Derived parameters for mean current velocity were friction velocity,

diffusion coefficients, rate constant of fertilization, and sperm-egg contact time. Derived parameters for friction velocity were diffusion coefficients and the rate constant of fertilization. To determine the effect of an increase in the proportion of males or females in a size class, I increased this proportion in one size class by 1% and decreased the proportion in each of the other size classes in proportion to the abundance in that class.

For population density and the proportions of adults, spawning adults, females, and sea urchins downstream of the nearest spawning male, a 1% increase did not alter the cumulative proportion of eggs fertilized by multiple males because rounding of the parameter derived from these basic parameters (distance to the nearest downstream spawning female, $r^{(n)}$) nullifies a small change in basic parameters. As an alternative approach, I determined sensitivity to this suite of parameters by increasing the distance between the nearest male and the downstream spawning female ($r^{(1)}$) in each subpopulation by one interval (0.05 m). For this analysis, the term $((v^{*}-v_k)/v_k)$ in equation [6.21] is not a constant (0.010) but varies among subpopulations (kelp beds: 0.026; grazing fronts: 0.200; transitional barrens: 0.067; post-transitional barrens: 0.077).

I estimated the proportion of spawning adults (c) based on the proportion of adult sea urchins that were partially spawned or spent on a single sampling date. Because there was a 1-month interval between that sample and a previous one, c reflects the proportion of adult sea urchins that had spawned over a month. As a result, I may overestimate c during the short time interval over which fertilizations were predicted to occur (maximum of 150 s, in kelp beds). To determine how a potential overestimate may affect the cumulative proportion of eggs fertilized by multiple males, I calculated the reduction in $\varphi_{j_i,k}^{(c)}$ when the proportion of adult spawners was reduced to 0.1 (or by 70%).

Current velocities in the shallow subtidal zone vary significantly in space and time. Because of the importance of this parameter to fertilization rates (Pennington 1985, Denny and Shibata 1989, Levitan and Young 1995), I estimated the cumulative proportion of eggs fertilized by multiple males at mean velocities of 0.05 m s^{-1} in kelp beds and 0.17 m s^{-1} in grazing fronts and barrens, the maximum values measured at a high flow site in Maine (Wahle and Peckham in press). I present the results of this analysis, in which I varied original parameter values approximately three-fold, as the reduction in $\varphi^{(C)}_{j_k,k}$. To directly assess the effect of differences in estimated mean current velocity and friction velocity among subpopulations, I also determined $\varphi^{(C)}_{j_k,k}$ for multiple males in kelp beds at the same current and friction velocity as in grazing fronts and barrens.

During analyses it became apparent that the use of multiple males dampened the effects of parameter changes on the cumulative proportion of eggs fertilized. To determine the maximum magnitude of these effects, I therefore repeated all sensitivity analyses using the cumulative proportion of eggs fertilized by a single (the nearest) male ($\varphi^{(C)}_{j_k,k}$, eq. [6.15] with m=1) as the model output.

My analysis indicated that sensitivity to small changes in parameters is highest for the number of eggs spawned per unit area, intermediate for the cumulative proportion of eggs fertilized by a single male, and lowest for the cumulative proportion of eggs fertilized by multiple males (Figs. 6.6, 6.7). Predicted fertilization rate of a single male or multiple males showed a high (E=0.82) or intermediate (E=0.38) sensitivity, respectively, to a change in friction velocity, intermediate (E=-0.40 to 0.40) or low (E=-0.19 to 0.18) sensitivities to changes in mean current velocity, the distance between the nearest male and the downstream spawning female, the proportion of

gonads that is spawned in males, and the proportion of body weight that is gonads in males (Fig. 6.6). Predicted fertilization rate of a single male or multiple males was relatively insensitive to a change in the proportion of males in any size class of adults (E=-0.08 to 0.02 or –0.03 to 0.01; Fig. 6.7). The number of eggs spawned per unit area was most sensitive (E=1.0) to increases in the proportion of gonads that is spawned in females and the proportion of body weight that is gonads in females (Fig. 6.6). Sensitivity was slightly lower (E=0.92) to changes in the proportions of females and adults, and to changes in population density. The proportion of females in a size class of adults (especially the two smallest size classes, Fig. 6.7) resulted in intermediate sensitivities (E=-0.41 to 0.16).

A 70% reduction in the proportion of spawning adults reduced fertilization rates by a single male or by multiple males by 28% or 12% respectively (i.e., to 57 or 83% fertilization respectively) in kelp beds, by 13% or 7% (to 81 or 91%) in grazing fronts, by 35% or 15% (to 46 or 76%) in transitional barrens, and by 31% or 10% (to 52 or 84%) in post-transitional barrens.

An approximately three-fold increase in current velocity reduced fertilization rates by a single male or by multiple males by 71% or 26% respectively (to 22 or 69% fertilization respectively) in kelp beds, by 37% or 17% (to 62 or 80%) in grazing fronts, by 69% or 36% (to 22 or 57%) in transitional barrens, and by 61% or 25% (to 30 or 70%) in post-transitional barrens. When mean current velocity and friction velocity in kelp beds were simultaneously raised to the same level as in grazing fronts and barrens (a four-fold increase), fertilization rates by one or by multiple males declined by 53% or 18% (to 36 or 76%).

Table 6.1: Summary of published data on population density, proportion of adults, mean and maximum adult size of sea urchins in kelp beds, grazing fronts, transitional and post-transitional barrens along the Atlantic coast of Nova Scotia.

Location	Sampling period	Population density (no. m^{-2})	Proportion of adults	Mean adult size (mm)	Maximum adult size (mm)	Source
Kelp beds						
St. Margaret's Bay	1973	230	0.02	32.5	35	Breen and Mann 1976b[a]
Boutilier Point	1977	29.5				Chapman 1981
Boutilier Point	10/1977-1/1980	0				Bernstein et al. 1981
Coyle Cove	6/1981	13	0.20	30.4	50	Miller 1985a
Little Duck Island	1992-1993	20	0.45	30.7	60	Scheibling et al. submitted[b]
Mill Cove	1992-1993	5	0.23	24.8	35	
Grazing fronts						
St. Margaret's Bay	1973		0.73	37.2	55	Breen and Mann 1976a
St. Margaret's Bay	1973	240	0.88	39.8	60	Breen and Mann 1976b
Clark's Harbour	1978/79	48	0.79	40.3	75	Wharton and Mann 1981[c]
Boutilier Point	1978	50				Bernstein et al. 1981[d]
Little Duck Island	1992-1993	256	0.95	43.1	65	Scheibling et al. submitted[b]
Mill Cove	1992-1993	84	0.73	34.8	55	

Table 6.1 (continued)

Location	Sampling period	Population density (no. m^{-2})	Proportion of adults	Mean adult size (mm)	Maximum adult size (mm)	Source
Transitional barrens						
St. Margaret's Bay	1973	75	0.54	36.1	55	Breen and Mann 1976b[c]
Boutilier Point	10/1977-1/1980	18				Bernstein et al. 1981[f]
Eagle Head	3/1983	24	0.48	34.9	50	Scheibling and Stephenson 1984
Eagle Head	3/1983	26	0.71	32.9	50	
Coyle Cove	6/1981	18	0.60	38.6	70	Miller 1985a
Little Duck Island	1992-1993	80	0.20	33.4	60	Scheibling et al. submitted[b]
Mill Cove	1992-1993	48	0.18	31.4	50	
Post-transitional barrens[g]						
Horse Island (2)	1973	80	0.22	31.4	45	Breen and Mann 1976b
Luke Island (5)	1973	110	0.04	30.3	35	
Northwest Cove (1)	5-9/1975	50				Lang and Mann 1976
Northwest Cove (3)	5-9/1975	70				
Northwest Cove (4)	5-9/1975	95				

Table 6.1 (continued)

Location	Sampling period	Population density (no. m^{-2})	Proportion of adults	Mean adult size (mm)	Maximum adult size (mm)	Source
Post-transitional barrens (continued)[g]						
Ingomar	1978/79	105.4	0.10	38.0	60	Wharton 1980a
Port Mouton	1978/79	107.9	0.13	35.4	60	
Blue Rocks	1978/79	61.9	0.04	30.0	40	
Northwest Cove	1978/79	67.5	0.13	30.8	50	
Mushaboom	1978/79	55.0	0.58	32.6	55	
Drum Head	1978/79	29.4	0.92	38.6	55	
Dover	1978/79	45.6	0.71	30.7	50	
Northwest Cove (1)	5-9/1975	53	0.64	33.4	50	
Northwest Cove (4)	5-9/1979	96	0.23	29.3	40	
Luke Island (5-6)	1973	112	0.05	30.5	35	
St. Margaret's Bay (8)	1975 (?)	43	0.09	31.9	45	
St. Margaret's Bay (11-12)	1970s	25	0.42	30.4	45	
Louse Head	8/1984	89.3	0.45	41.0	60	Scheibling 1986
Cape Mocodome	8/1984	49.8	0.59	46.5	70	

[a] Population density and proportion of adults from this study are not included in calculating the means of these two parameters as they appear to be distorted by a heavy recruitment event.

[b] Data from 1994 and 1995 are not used because of a mass mortality in fall 1993 at Little Duck Island.

[c] Site 2b in this study.

[d] Only data from July and December 1978 are used because densities in other months do not indicate the existence of a grazing front.

[e] Data collected 5 m from the edge of the kelp bed.

[f] Data averaged from exposed and hidden sea urchins collected during the day.

[g] Numbers in brackets indicate years since kelp bed destruction.

Table 6.2: Basic and derived parameters used to calculate sperm concentration, proportion of eggs fertilized, and eggs spawned per unit area in kelp beds, grazing fronts, transitional and post-transitional barrens.

Parameter	Symbol	Units	Kelp beds	Grazing fronts	Transitional barrens	Post-transitional barrens
Basic parameters						
Proportion of adults in size class	p					
20-25 mm, j=22.5 mm			0.409	0.123		
25-30 mm, j=27.5 mm			0.243	0.133	0.288	0.338
30-35 mm, j=32.5 mm			0.161	0.172	0.323	0.232
35-40 mm, j=37.5 mm			0.059	0.157	0.206	0.165
40-45 mm, j=42.5 mm			0.089	0.146	0.097	0.128
45-50 mm, j=47.5 mm			0.026	0.131	0.057	0.077
50-55 mm, j=52.5 mm			0.007	0.084	0.014	0.035
55-60 mm, j=57.5 mm			0.006	0.033	0.010	0.016
Population density (SE)	ρ	no. m^{-2}	14 (5.3)	136 (46.4)	41 (10.1)	71 (6.4)
Proportion of adults (SE)	a		0.29 (0.09)	0.82 (0.04)	0.45 (0.07)	0.33 (0.07)
Proportion of spawning adults	b		0.33	0.33	0.33	0.33
Proportion of females	f		0.50	0.50	0.50	0.50
Proportion of sea urchins downstream of						
1st male	$c^{(1)}$		0.105	0.229	0.149	0.158
2nd male	$c^{(2)}$		0.082	0.192	0.119	0.128
3rd male	$c^{(3)}$		0.071		0.104	0.111
4th male	$c^{(4)}$		0.064			0.102

Table 6.2 (continued)

Parameter	Symbol	Units	Kelp beds	Grazing fronts	Transitional barrens	Post-transitional barrens
Mean current velocity at 0.1 m above substratum	$\bar{u}$	m s^{-1}	0.014	0.056	0.056	0.056
Friction velocity (% of $\bar{u}$ at 0.1 m above substratum)	u_*	m s^{-1}	0.0016 (11.76)	0.0077 (13.76)	0.0077 (13.76)	0.0077 (13.76)
Proportion of total body weight that is gonads in males/females	I					
20-25 mm, j=22.5 mm			0.008 / 0.008	0.010 / 0.010		
25-30 mm, j=27.5 mm			0.030 / 0.030	0.038 / 0.038	0.025 / 0.025	0.009 / 0.009
30-35 mm, j=32.5 mm			0.085 / 0.085	0.098 / 0.098	0.062 / 0.062	0.026 / 0.026
35-40 mm, j=37.5 mm			0.157 / 0.205	0.128 / 0.159	0.085 / 0.112	0.047 / 0.062
40-45 mm, j=42.5 mm			0.157 / 0.205	0.128 / 0.159	0.085 / 0.112	0.047 / 0.062
45-50 mm, j=47.5 mm			0.157 / 0.205	0.128 / 0.159	0.085 / 0.112	0.047 / 0.062
50-55 mm, j=52.5 mm			0.157 / 0.205	0.128 / 0.159	0.085 / 0.112	0.047 / 0.062
55-60 mm, j=57.5 mm			0.157 / 0.205	0.128 / 0.159	0.085 / 0.112	0.047 / 0.062
Proportion of gonads that is spawned in males/females	C		0.65 / 0.70	0.65 / 0.72	0.67 / 0.77	0.67 / 0.77
Derived parameters						
Weighted mean adult size	j'	mm	29.1	37.8	34.2	34.9
Sperm release rate (weighted)	Q	no. s^{-1}	1.9×10^7	2.9×10^7	2.6×10^7	2.7×10^7
Rounded (exact) distance between spawning female and upstream spawning male						
nearest male	$r^{(1)}$	m	1.90 (1.91)	0.25 (0.24)	0.75 (0.74)	0.65 (0.64)
2nd nearest male	$r^{(2)}$	m	3.25 (3.25)	0.40 (0.400	1.25 (1.24)	1.05 (1.06)
3rd nearest male	$r^{(3)}$	m	4.35 (4.36)		1.65 (1.65)	1.45 (1.43)
4th nearest male	$r^{(4)}$	m	5.35 (5.35)			1.75 (1.74)

Table 6.2 (continued)

Parameter	Symbol	Units	Kelp beds	Grazing fronts	Transitional barrens	Post-transitional barrens
Derived parameters (continued)						
Diffusion coefficient of gamete plume at $r_k^{(1)}$ along horizontal/ vertical axis[a]	$\alpha(x)_y$ / $\alpha(x)_z$		0.685 / 0.428	1.747 / 1.092	1.008 / 0.630	1.083 / 0.677
Rate constant of fertilization	β	$mm^3\ s^{-1}$	4.51×10^{-3}	2.11×10^{-2}	2.11×10^{-2}	2.11×10^{-2}
Sperm-egg contact time	t	s	3.57	0.89	0.89	0.89

[a] Because the diffusion coefficients change with downstream distance (see eq. [6.10]), I present $\alpha(x)_y$ and $\alpha(x)_z$ only for one distance, namely $r_k^{(1)}$.

Fig. 6.1. Map of Nova Scotia showing the extent of mass mortalities of *Strongylocentrotus droebachiensis* during 1980-1983 and in 1995. Near-complete mass mortality is indicated by black bars, which also denote the extent of the shallow subtidal zone over which the number of eggs spawned and total zygote production is predicted. Gaps between bars indicate areas where sea urchins had been eliminated by disease in previous years. (Adapted from Scheibling and Hennigar 1997)

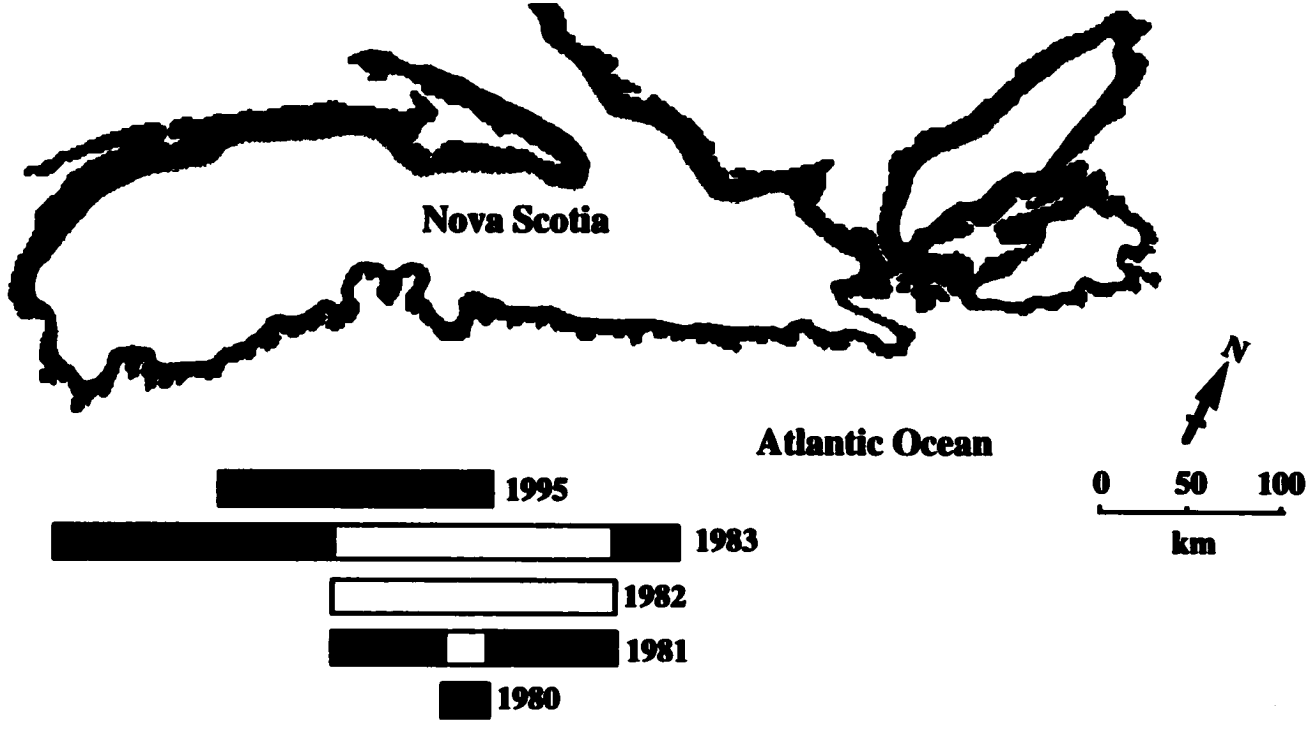
Nova Scotia
Atlantic Ocean
N
0
50
100
km
1995
1983
1982
1981
1980

Figure 6.1

Fig. 6.2. Sperm concentration in kelp beds, grazing fronts, transitional, and post-transitional barrens as a function of downstream distance from the spawning male.

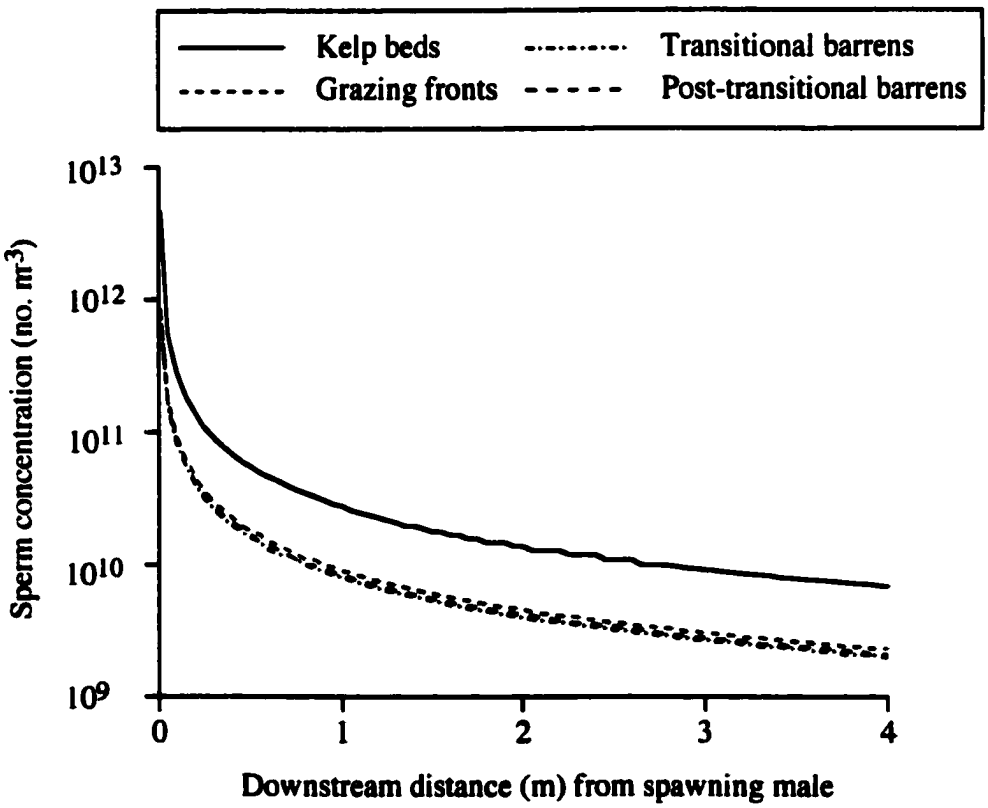
Kelp beds
Transitional barrens
Grazing fronts
Post-transitional barrens
Sperm concentration (no. m-3)
10^13
10^12
10^11
10^10
10^9
0
1
2
3
4
Downstream distance (m) from spawning male

Figure 6.2

Fig. 6.3. Predicted proportion of eggs fertilized by a) one male, and b) multiple (n) males of weighted mean size in kelp beds (n=4), grazing fronts (n=2), transitional (n=3), and post-transitional barrens (n=4) as a function of downstream distance from the spawning male closest to the downstream female. Arrows mark the location of the female (open arrow) and the point at which the maximum cumulative proportion of eggs is fertilized (closed arrow).

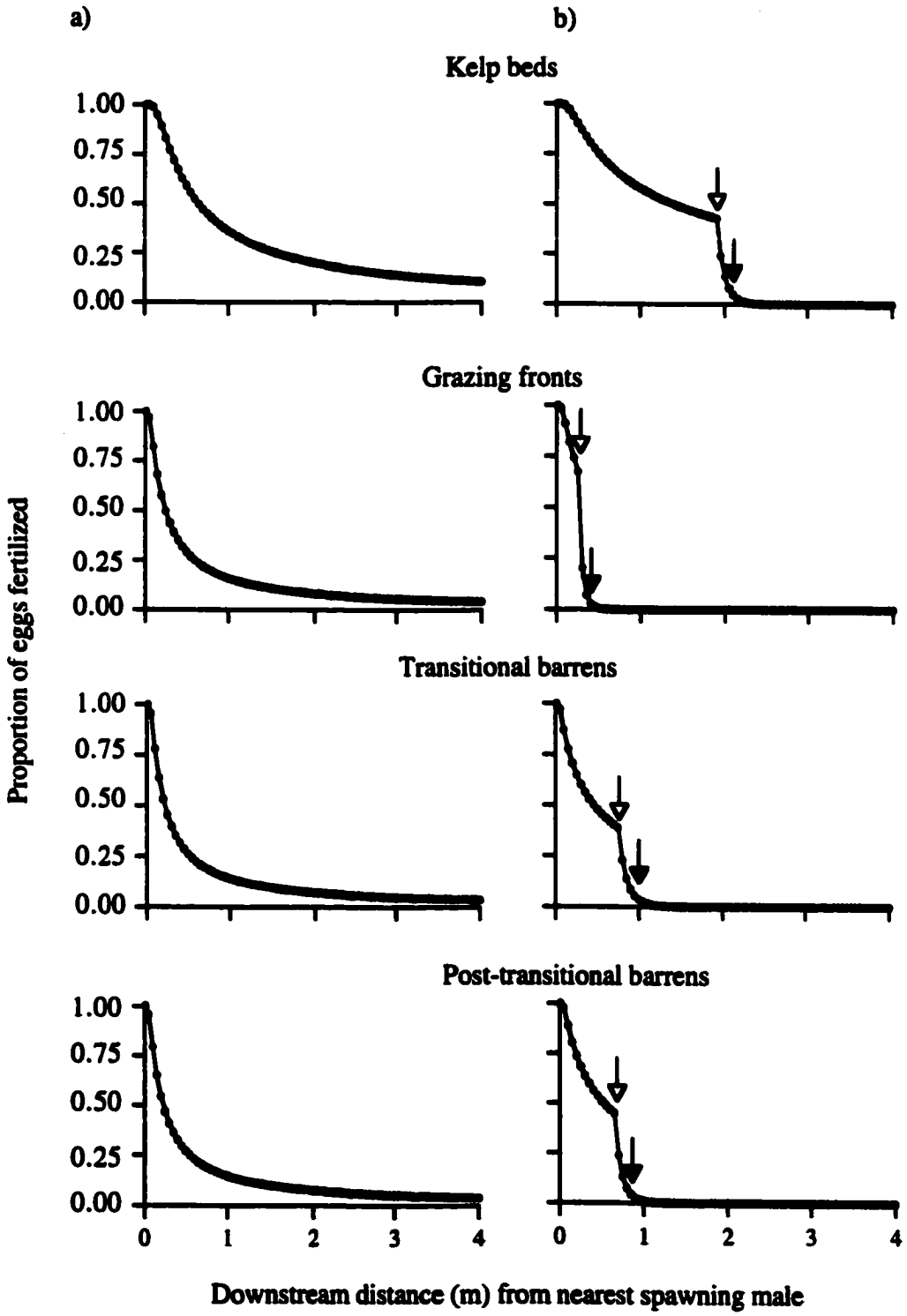
a)
b)
Kelp beds
Grazing fronts
Transitional barrens
Post-transitional barrens
Proportion of eggs fertilized
1.00
0.75
0.50
0.25
0.00
0
1
2
3
4
Downstream distance (m) from nearest spawning male

Figure 6.3

Fig. 6.4. Number (m^{-2}) of eggs spawned (hatched bars) and zygotes produced (grey bars) in kelp beds (KB), grazing fronts (GF), transitional (TB) and post-transitional (PB) barrens along the Atlantic coast of Nova Scotia.

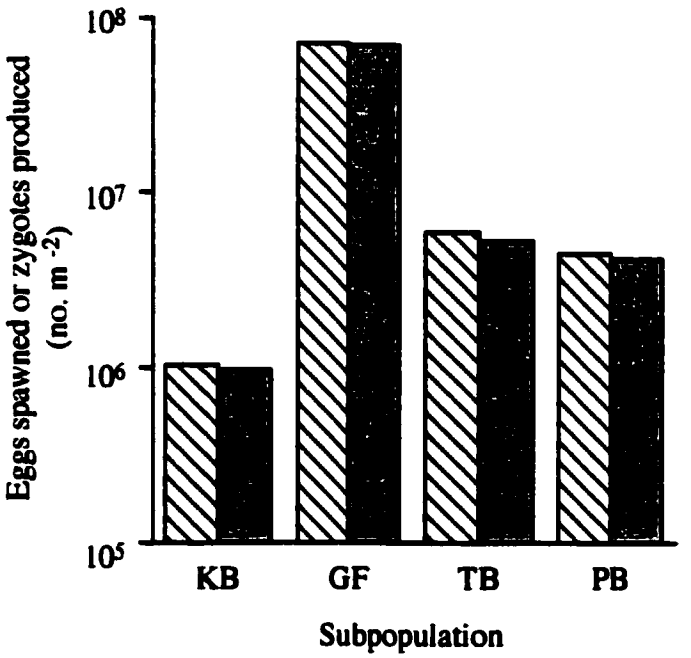
Eggs spawned or zygotes produced
(no. m -2)
10^8
10^7
10^6
10^5
KB
GF
TB
PB
Subpopulation

Figure 6.4

Fig. 6.5. Number of zygotes produced in kelp beds, grazing fronts, transitional and post-transitional barrens on a coastal scale, and total zygote production in the entire subtidal zone (bold line, which partly overlaps with lines indicating zygote production in kelp beds and post-transitional barrens), as the community moves from the established kelp bed via the transition to the barrens state over time.

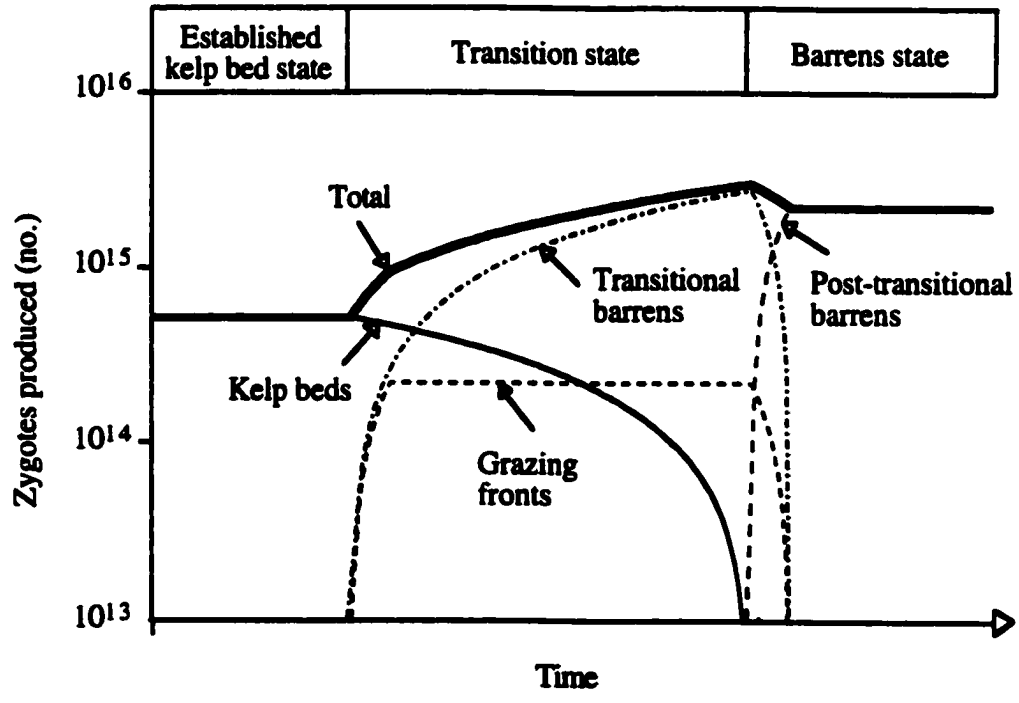
Established kelp bed state
Transition state
Barrens state
10^{16}
10^{15}
10^{14}
10^{13}
Zygotes produced (no.)
Total
Transitional barrens
Post-transitional barrens
Kelp beds
Grazing fronts
Time

Figure 6.5

Fig. 6.6. Mean (±SE) sensitivity of the cumulative proportion of eggs fertilized by a single male (grey bars) or by multiple males (black bars) or of the number of eggs spawned per unit area (open bars) to small changes in basic parameters. Means are based on sensitivities calculated in the four subpopulations (kelp beds, grazing fronts, transitional and post-transitional barrens). Proportional sensitivities shown here are equal to the proportional change in a model output when a parameter is increased by 1% (except for the distance between the nearest male and the downstream spawning female which is increased by 2.6-7.7%).

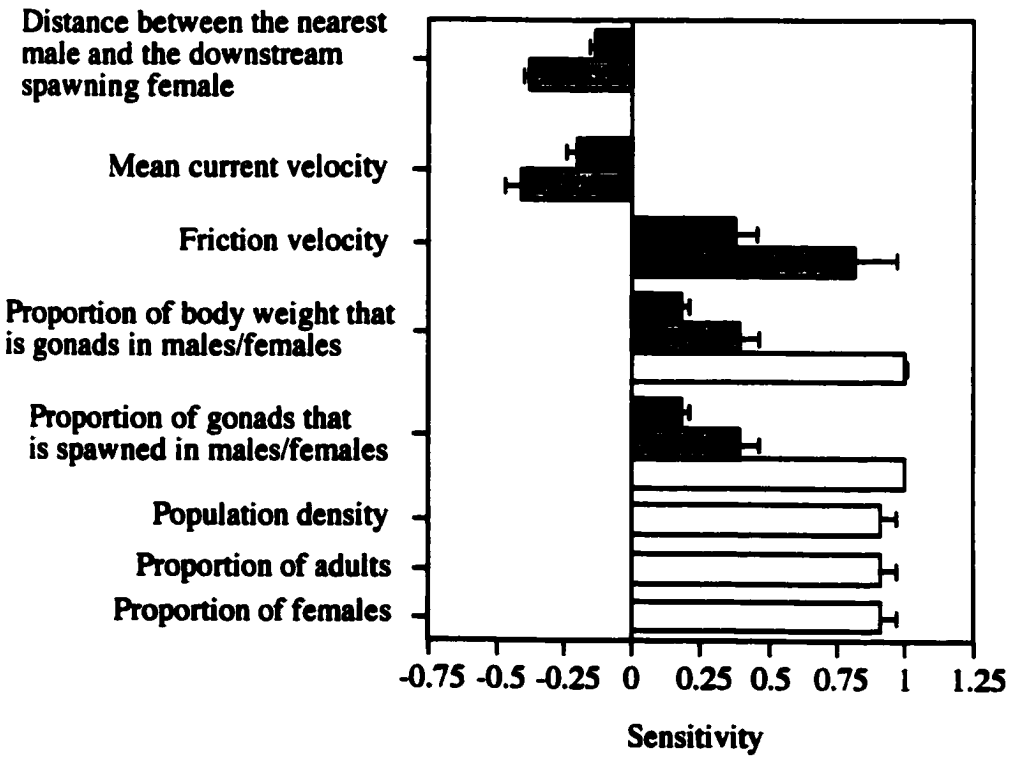
Distance between the nearest male and the downstream spawning female
Mean current velocity
Friction velocity
Proportion of body weight that is gonads in males/females
Proportion of gonads that is spawned in males/females
Population density
Proportion of adults
Proportion of females
-0.75 -0.5 -0.25 0 0.25 0.5 0.75 1 1.25
Sensitivity

Figure 6.6

Fig. 6.7. Mean (±SE) sensitivity of the cumulative proportion of eggs fertilized by a single male (grey bars) or by multiple males (black bars) or of the number of eggs spawned per unit area (open bars) to a small change in the proportion of males or females, respectively, in a size class (represented by its mid-point) of adults. Means at 22.5 mm are based on sensitivities calculated in kelp beds and grazing fronts, while means at all other sizes are based on sensitivities calculated in all four subpopulations (kelp beds, grazing fronts, transitional and post-transitional barrens). Proportional sensitivities shown here are equal to the proportional change in the number of zygotes produced when the proportion of males or females is increased by 1%.

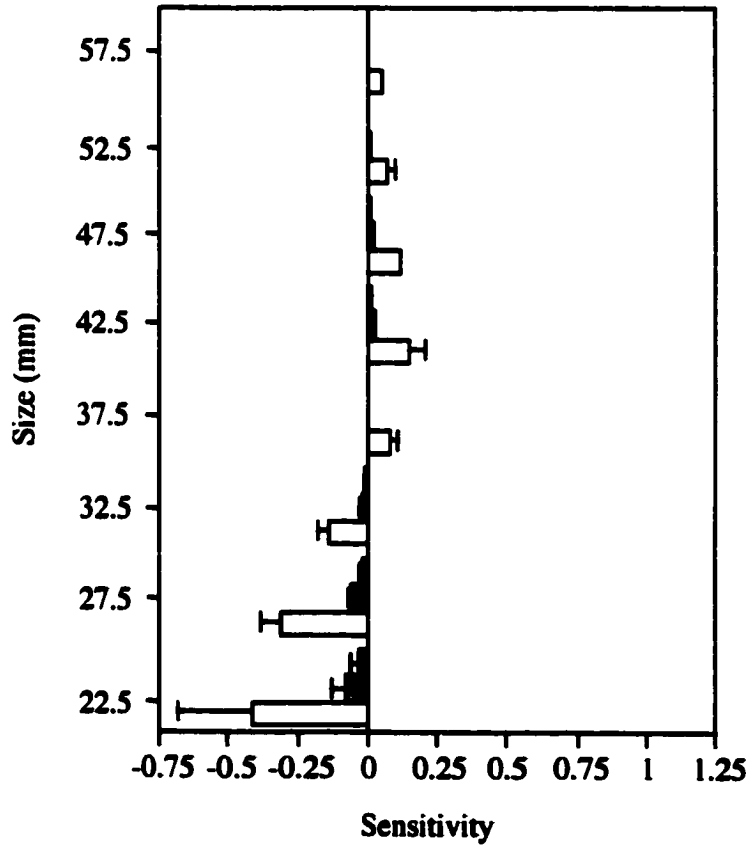
57.5
52.5
47.5
42.5
37.5
32.5
27.5
22.5
Size (mm)
-0.75 -0.5 -0.25 0 0.25 0.5 0.75 1 1.25
Sensitivity

Figure 6.7

DISCUSSION

Model assumptions and parameterization

The accuracy of my model predictions depends both on the validity of my assumptions and the accuracy of parameters that I estimated. In what follows, I consider the validity of several important assumptions and whether they are equally valid for all subpopulations. This assessment will indicate the extent to which my predictions are accurate in an absolute or relative (i.e., among subpopulations) sense. I also consider the accuracy of certain estimates of model parameters and the effect of omitting deeper-water populations from my models.

I estimate distances between multiple spawning males and the nearest downstream spawning female ($r^{(n)}$) using an equation that calculates expected nearest neighbour distances (r) based on a random dispersion of individuals. In nature, this assumption may be violated because sea urchins are usually aggregated in kelp beds and barrens (pers. obs.), and regularly spaced due to close packing of individuals within grazing fronts. My method of calculating $r^{(n)}$, however, excludes a large proportion of the population [i.e., juveniles, males, non-spawning individuals, and those not downstream of male(s)]. If I assume that sea urchins are randomly dispersed with respect to maturity, gender, reproductive stage, or current direction, this reduction in numbers would tend to make the dispersion pattern of the selected population approach a random distribution at the scales relevant to my study. As long as my estimate of the distance between the nearest male and the downstream spawning female ($r^{(1)}$) approximates the true distance, my estimate of zygote production likely will be reliable because this male fertilizes most of the eggs and the model output is not very sensitive to changes in $r^{(1)}$.

In the shallow subtidal zone, current velocity and direction vary in space and time as different types of currents (tidal or wind driven) interact with one another. Such changes in the hydrodynamic regime can affect my model predictions which are based on constant flow conditions. However, because my models predict cumulative fertilization rates over brief periods of time, fluctuations in current velocity or direction that happen over larger time scales will not affect my results. If the hydrodynamic regime fluctuates rapidly, predicted fertilization rates in different subpopulations are likely to be incorrect, in both a relative and absolute sense, because the magnitude of fluctuations would probably be dissimilar in the different habitats, especially at the extreme ends of the depth range under consideration. The mean current and friction velocities I use should be appropriate in general, and adequately reflect differences in these parameters among subpopulations. Sensitivity analysis indicates that, for an approximately three-fold increase in current velocity, fertilization rates decline by 17-36% in absolute terms. Therefore, whenever the hydrodynamic regime differs considerably from the estimates used here, my model predictions will be inaccurate in an absolute sense.

The model I use to calculate sperm concentration assumes that gametes disperse in a plume (Denny 1988) and that sperm and eggs travel together downstream. Eggs of *Strongylocentrotus droebachiensis* are negatively buoyant (pers. obs.) and may settle out of a plume, thus reducing their exposure to sperm. Consequently, I may overestimate the proportion of eggs fertilized, especially in kelp beds where current velocity and turbulence are low. Also, in flume studies with the sea urchins *Tripneustes gratilla*, *Echinometra mathaei*, and *Colobocentrotus atratus*, Thomas (1994) showed that at mainstream velocities of <0.13 m s^{-1} gametes are not evenly dispersed, but form clumps or strings. These structures may enhance fertilization rates by trapping other

gametes or resisting dispersion. If *S. droebachiensis* also produces gamete clumps or strings at low flow, my use of a gamete dispersion model may underestimate fertilization rates under these conditions (e.g., in kelp beds).

Sensitivity analysis indicates that the accuracy of my predictions also depends largely on the accuracy of estimates of population parameters. I estimate these parameters based on several studies of populations of *S. droebachiensis* along the Atlantic coast of Nova Scotia. Although absolute parameter values may vary over time and space within subpopulations, the rank order of those values among subpopulations is likely correct. Therefore, my model should accurately predict the relative zygote production of different subpopulations.

The approach I use to estimate the proportion of adult spawners may overestimate that parameter. In nature, spawning of *S. droebachiensis* has only been observed outside the major spawning season when it involved a small percentage of the population (up to 15%, Keats et al. 1987; 5%, Pearse et al. 1988). Spawning by a low number of individuals has previously been reported for other sea urchins (Pennington 1985, Levitan 1988b) although 'mass spawning' has been observed in *S. purpuratus*, one unidentified sea urchin (Pennington 1985), and *Evechinus chloroticus* (Lamare and Stewart 1998). When I reduce the proportion of spawning adults to 0.1 (i.e., to a proportion similar to what has been observed in the field), the predicted fertilization rate by multiple males decreases by 7-15%, suggesting that an overestimate of this parameter would not markedly alter model outputs.

My calculations of zygote production and contribution of different subpopulations to the overall zygote pool are limited to the shallow subtidal zone above 15 m depth. Although the abundance and size of *Strongylocentrotus droebachiensis* declines considerably at greater depths (Propp 1977, Logan et al. 1988, Martin et al.

1988), no information is available on the reproductive output or demography of these populations. Also, no measures exist on the extent of hard substratum below 15 m depth along the Atlantic coast of Nova Scotia. Therefore, I cannot evaluate the potential of deeper-water populations for zygote production. While populations below 30 m are likely to have a low reproductive output due to a lack of macroalgal food, those at intermediate depths (15-30 m) may produce a considerable number of zygotes as their food supply is augmented by drift algae from the shallows. It is thus likely that I am underestimating total zygote production in the subtidal zone, especially during the established kelp bed state when the reproductive potential of deeper-living populations may be enhanced by inputs of drift algae (Scheibling et al. submitted).

In summary, my evaluation of assumptions and sensitivity analyses suggest that my estimates of zygote production in subpopulations of *S. droebachiensis* are most influenced by variability in hydrodynamic conditions. In general, my predictions should be correct in a relative sense although absolute numbers may be inaccurate, particularly if I have overestimated fertilization rates. Because my predicted fertilization rates are close to the maximum, I have probably overestimated, rather than underestimated, zygote production on an areal basis. Total zygote production on a coastal scale, however, may be underestimated due to the omission of sea urchins at depths >15 m. To improve model predictions, we need to measure nearest-neighbour distances in subpopulations, determine gamete dispersion in *S. droebachiensis* under different flow conditions, obtain a better estimate of spawning synchrony, and collect demographic data on sea urchin populations at depths ~15-30 m. A more accurate estimate of mean current velocity based on measurements collected over a wide range of conditions would indicate the frequency of the mean velocities I estimated. To validate predicted fertilization rates, carefully designed field experiments are required.

Variation in fertilization rate

Sensitivity analysis indicates that the predicted fertilization rate is most influenced by the hydrodynamic regime (which determines sperm dilution rates), distance between a spawning male and the nearest downstream spawning female, and male gonad output. Each of these parameters, varies markedly among subpopulations of *Strongylocentrotus droebachiensis* (Table 6.2), resulting in differences in predicted fertilization rates. Fertilization rate is highest (97%), with the fewest (two) spawning males, for sea urchins in grazing fronts where the current regime is moderate, and large adults with a relatively high gonad output occur at extremely high densities (i.e., at small distances between spawners). In barrens, where the hydrodynamic regime is presumed to be similar to that along grazing fronts, but adult density and gonad output are lower, three (transitional barrens) or four (post-transitional barrens) spawning males also achieve high fertilization rates (89 and 93%, respectively). Thus, under the same hydrodynamic conditions, the predicted fertilization rate increases with the density of spawners. This pattern is consistent with field fertilization experiments on sea urchins (Pennington 1985, Levitan 1991, Levitan et al. 1992), a coral (Brazeau and Lasker 1992), and an ascidian (Yund and McCartney 1994, Yund 1995, 1998), and fertilization rates measured during natural spawning events of a starfish (Babcock and Mundy 1992) and a coral (Coma and Lasker 1997). In kelp beds, a weak current regime reduces sperm dilution rate relative to grazing fronts and barrens. The weak flow counteracts the low density of adults, resulting in high fertilization rates if sperm is pooled from four males. A positive effect of a low flow regime has previously been demonstrated in field fertilization experiments involving sea urchins (Pennington 1985, Levitan et al. 1992, Wahle and Peckham in press).

According to my application of the fertilization kinetics model, multiple males fertilize >89% of spawned eggs in all subpopulations. In contrast, most experimental studies involving sea urchins have measured fertilization rates that are much lower (4-61%; Levitan 1991, Levitan et al. 1992, Wahle and Peckham in press; but see Pennington 1985 for rates of up to 80%). Field experiments have associated artifacts (Levitan 1995) such as induced spawning of individuals, confinement of eggs in mesh bags, or release of sperm from syringes, which limit the degree to which they mimic natural spawning events. On the other hand, my theoretical approach is dependent on the validity of various assumptions about physical and biological processes, and on accurate model parameterization. Because of the uncertainties inherent in both empirical and theoretical approaches, I do not attempt to reconcile my predicted fertilization rates with those measured in the field.

Variation in the number of eggs spawned per unit area

The density of adults also determines differences among subpopulations of *Strongylocentrotus droebachiensis* in the predicted the number of eggs spawned per m^2. Other important parameters which influence egg spawning (as indicated by sensitivity analysis), and which vary among subpopulations, are related to the size and gonad output of females. Sea urchins in grazing fronts release the largest number of eggs per unit area because extremely high densities of large females spawn a high proportion of their relatively large gonads. In transitional and post-transitional barrens, where females are less dense and smaller, and have a smaller gonad output than in grazing fronts, the predicted number of eggs spawned per unit area is about one order of magnitude lower than in fronts. In kelp beds, where relatively small females occur at very low densities, the predicted number of eggs spawned per unit area drops to about one fifth of that

spawned in barrens. In a study of variation in the gonad output of *S. droebachiensis* at different depths (0-18 m), Keats et al. (1984) estimated that sea urchins in kelp beds produced a gamete biomass per unit area (12 g m^{-2}) that was similar to or less than that produced by sea urchins at higher densities in barrens at greater depths (9-49 g m^{-2}).

Variation in zygote production

Because predicted fertilization rates are similar, variation in zygote production per unit area among subpopulations of *Strongylocentrotus droebachiensis* primarily reflects differences in the number of eggs spawned. The contribution of each subpopulation to total zygote production on a coastal scale is determined largely by the extent of the subtidal zone that a given subpopulation occupies. Sea urchins in grazing fronts, which produce the largest number of zygotes per unit area, but occupy <1% of the rocky subtidal zone, generally produce fewer zygotes than sea urchins in other subpopulations. For sea urchins in kelp beds and transitional barrens, zygote production decreases or increases respectively, as kelp beds are destroyed by grazing fronts and replaced with barrens during the transition state. Because of the marked difference in the number of eggs spawned by sea urchins in these two habitats, predicted zygote production in kelp beds exceeds that in barrens only in the early stages of the transition state. In post-transitional barrens which occupy the entire subtidal zone, zygote production is ~20% lower than in transitional barrens, but four times higher than in the former kelp beds. Total zygote production by all subpopulations combined increases approximately six-fold from the established kelp bed state to the late transition state when sea urchins in transitional barrens and grazing fronts occupy their respective maximum areas. In the barrens state, total zygote production decreases by approximately

25% relative to the late transition state as sea urchins in grazing fronts disperse and transitional barrens are replaced by post-transitional barrens.

Variation in zygote production and sea urchin population dynamics

To assess the role that variation in zygote production may play in the population dynamics of *Strongylocentrotus droebachiensis* along the Atlantic coast of Nova Scotia, I compare this variation with natural variation in settlement. On a coastal scale, I estimate that total zygote production in the shallow subtidal zone differs six-fold among community states that include adult sea urchins. Because no zygotes are produced in the shallow subtidal zone after a sea urchin mass mortality, variation in total zygote production is even greater if I include the developing kelp bed state (from 0 to ~10^{15} m^{-2}). However, settlement can occur shortly after a sea urchin die-off (Miller 1985a, Scheibling 1986, Raymond and Scheibling 1987, Scheibling and Raymond 1990) and can vary interannually by more than an order of magnitude during the transition from kelp beds to barrens (Balch and Scheibling unpubl. data), when my model predicts a more gradual increase. This discrepancy between the temporal patterns of estimated zygote production and observed settlement rates suggests that settlement of *S. droebachiensis* in the shallow subtidal zone is not primarily determined by zygote production within that zone. Other important factors may include advective transport of larvae to and from the region (Scheltema 1986, Shanks 1995), and sea temperature which regulates the rate of larval development and hence survival in the plankton (Hart and Scheibling 1988a).

Understanding the relative importance of subpopulations as contributors to the overall zygote pool is critical to evaluating the potential impact of the commercial sea urchin fishery on populations of *S. droebachiensis* off Nova Scotia. Because this

fishery targets the large individuals in grazing fronts, which according to my estimates contribute only 4-7% of all zygotes produced during the transition state, harvesting will not directly alter zygote production to a large degree. However, over-exploitation of grazing fronts may indirectly affect population dynamics in various ways. Because settlement of sea urchins is higher (Miller 1985a, Scheibling 1986, Leinaas and Christie 1996, Balch and Scheibling 1998, Balch et al. 1998), and post-settlement mortality is lower (Rowley 1989, 1990), in barrens than in kelp beds, overharvesting of fronts may limit the expansion of barrens and hence the recruitment potential of sea urchins in the shallow subtidal zone. Furthermore, destructive grazing at fronts increases the production of drift algae which may enhance gonad production in transitional barrens (Scheibling et al. submitted). Therefore, the importance of sea urchins in fronts extends beyond their contribution to the overall zygote pool, and removal of this subpopulation may ultimately jeopardize a sustainable harvest of sea urchins.

My modelling approach also can be used to evaluate whether enough zygotes are produced during the established kelp bed state to initiate a population outbreak of sea urchins that would lead to destructive grazing. I will address this issue by estimating the number of zygotes required to initiate an outbreak and compare this to the predicted number of zygotes produced in kelp beds during the established kelp bed state. Breen and Mann (1976a) estimated that a threshold biomass of sea urchins of ~2 kg m^{-2} is required for the formation of grazing fronts and the initiation of destructive grazing (see also Scheibling et al. submitted). This equates to ~80 individuals of 38 mm test diameter, the mean adult size in grazing fronts (Table 6.2). Rowley (1990) found that ~90% of settlers of *S. purpuratus* died within 40 days of settling. If I assume a similar mortality during the 4-5 years that sea urchins in kelp beds take to grow to 38 mm (Chapter 3), this gives me a cumulative post-settlement mortality rate of 99%.

Therefore, a settler density of 8000 m^{-2} is required to achieve the adult density necessary to initiate destructive grazing. If I assume a mortality rate of 99.9% during the extended planktonic phase of *S. droebachiensis* (51-152 days, Strathmann 1978), a settler density of 8000 m^{-2} would require the production of 8.0 x 10^6 zygotes m^{-2}. This is about one order of magnitude greater than the predicted zygote production in the established kelp bed state (9.6 x 10^5 m^{-2}).

As discussed above, settlement in the shallow subtidal zone under consideration is in part due to the import of larvae from other areas. Using published data collected during the developing kelp bed state, I can obtain a rough estimate of the number of larvae imported into the region when kelp beds dominate the subtidal zone. After a mass mortality in the fall of 1983, when there was no longer any zygote production in the shallow subtidal zone, Scheibling and Raymond (1990) recorded a cohort of recruits in late August 1984 (i.e., 1-2 months after the peak settlement period off Nova Scotia, Balch et al. 1998) which increased the density of juvenile sea urchins from ~28 to 72 m^{-2}. Thus, I estimate the density of the 1984 recruits as 44 m^{-2}, and, assuming an early post-settlement mortality rate of 90% (Rowley 1990), the initial density of settlers as ~440 m^{-2}. Assuming a 99.9% mortality rate in the plankton, I estimate that a production of ~4.4 x 10^5 zygotes m^{-2} would be required to yield that many imported settlers. Using this value to estimate imported zygote production and combining it with my estimate of zygote production in the established kelp bed state (but not accounting for zygote export to other regions), I get ~1.4 x 10^6 zygotes that may be available per m^2 of the subtidal region under consideration. This is about one-fifth of the number required to initiate a population outbreak, which suggests that total annual zygote production from all regions during the established kelp bed state is not large enough to lead to an increase in sea urchin numbers in the shallow subtidal zone.

To assess whether zygote production during the established kelp bed state may lead to an increase in population density over several years, I apply the same mortality rates to my estimate of total zygote production from all regions (1.4×10^6 m^{-2}). Thus, each settlement event would add, after 4-5 years of growth (Chapter 3), 14 adults per m^2 to the subpopulation in kelp beds, and 5-6 successive events would be required to increase population density to a level where destructive grazing could occur. However, because of the episodic nature of recruitment of *S. droebachiensis* along the coast of Nova Scotia (Raymond and Scheibling 1987, Scheibling and Raymond 1990, Balch and Scheibling unpubl. data), a decade or more may elapse between an initial settlement event and the establishment of a sea urchin population that is capable of initiating destructive grazing. This time scale is inconsistent with the observation that, following mass mortalities of *S. droebachiensis* in 1981-1983, destructive grazing of recently established kelp beds may have begun by the mid to late 1980s in some localities (Scheibling et al. submitted). Furthermore, because sea urchins in kelp beds are often small even a decade or more after a mortality event (Scheibling et al. submitted, Chapter 3), it is unlikely that the large sea urchins which aggregate in grazing fronts come from within kelp beds. Therefore, my analysis lends support to the hypothesis that fronts develop as sea urchins migrate from deeper waters into the shallows where they accumulate at the edge of a kelp bed to form fronts (Scheibling et al. submitted). Zygote production in kelp beds, however, may contribute to a sea urchin outbreak by supplying settlers to deep-water barrens and expanding these populations.

Conclusions

Several of the factors that determine the reproductive success of sea urchins (e.g., population density, proportion of adults, gamete output, hydrodynamic regime)

vary among subpopulations in kelp beds, grazing fronts, transitional and post-transitional barrens. Because of this variation, subpopulations differ in their contribution to the total pool of zygotes produced in the region where sea urchin mass mortalities occur. As the shallow subtidal ecosystem moves through the different community states, and subpopulations replace one another, total zygote production varies by several orders of magnitude. My model predictions, when compared to empirical data on patterns of *S. droebachiensis* abundance, suggest that temporal variation in zygote production alone does not explain sea urchin outbreaks or large fluctuations in settlement along this coast. A clearer resolution of the importance of variation in zygote production to population dynamics of *S. droebachiensis*, relative to other biological and physical factors, requires further study. For example, longitudinal studies spanning different community states may elucidate the relationship between patterns of zygote production and the frequency and magnitude of settlement events. Genetic analysis of larvae and settlers also would be helpful in determining the origins of larvae settling along this coast (Palumbi 1995, Medeiros-Bergen et al. 1995). Finally, investigation of deep-water populations is needed to assess their contribution to the larval pool and their potential to initiate destructive grazing of kelp beds through migration.

Chapter 7: General discussion

My thesis work has focussed largely on the effects of diet on reproductive processes in *Strongylocentrotus droebachiensis*. The main goal of this research was to elucidate the role of temporal and spatial variation in zygote production in the population dynamics of this species along the Atlantic coast of Nova Scotia. Sea urchins in kelp beds and grazing fronts exhibit increased gonadal production relative to those in barrens because they consume a higher quality diet (Chapter 2). However, differences in diet among habitats do not influence the quality of gonads, the annual nature of the reproductive cycle, regional spawning synchrony, or sexual differences in gonad index. This suggests that these reproductive traits are governed by factors other than food, such as temperature, photoperiod (Gonor 1973a), or phytoplankton blooms (Himmelman 1975). In contrast to reproduction, diet had a small effect on the growth rate of adults, which was marginally greater in kelp beds and grazing fronts than in barrens at one of two sites (Chapter 3).

Like adults, juvenile sea urchins in kelp beds and grazing fronts consume a higher quality diet than those in barrens (Chapter 3). Because juveniles channel most of their energy into growth, this difference in nutrition results in a faster growth rate in kelp beds and grazing fronts. A strong positive effect of diet quality and quantity on juvenile growth was also evident in my laboratory feeding experiment (Chapter 4), which indicated that juveniles in kelp beds may reproduce at a younger age than those in barrens, and produce larger gonads at first reproduction. As young sea urchins are abundant in kelp beds and barrens (Chapter 3), the age at first reproduction will markedly influence the proportion of reproductive individuals in these habitats. This experiment furthermore indicated that juveniles in severely food-limited environments,

such as deep-water barrens, may delay first reproduction and have a low reproductive potential (Chapter 4).

Diet quality and quantity strongly influenced the quantity of gonads produced by young (Chapter 4) or older adults (Chapter 2), but had little effect on the quality of gonads or offspring (Chapter 5). Effects of parental nutrition on larval growth, development, rate of metamorphosis, and post-metamorphic size were small, both in absolute terms and when compared to the effect of larval nutrition. However, my results indicate that when larval food in the plankton is abundant, larvae from adults in kelp beds may metamorphose sooner than those from adults in barrens, suggesting a difference in larval quality.

To determine the role that temporal and spatial variation in zygote production may play in the population dynamics of *S. droebachiensis*, I combined observations and experimental results with other published or unpublished data to develop and parameterize models of fertilization kinetics and egg production (Chapter 6). The fertilization rate predicted by my model was most influenced by adult density, gamete output, and the hydrodynamic regime. The predicted number of eggs spawned per unit area was also highly influenced by adult density and gamete output, as well as by female size. The model showed that sea urchins in kelp beds produce a much smaller number of zygotes per unit area than those in grazing fronts and barrens despite a high individual reproductive rate (Chapter 5). I estimated that total zygote production in the shallow rocky subtidal zone (<15 m) increased six-fold during the transition from kelp beds to barrens (i.e., during a sea urchin outbreak). However, because no zygotes are produced in that zone following a sea urchin mass mortality, variation in total zygote production at decadel time scales is much greater. A comparison of temporal variation in both estimated zygote production and observed settlement patterns (Miller 1985, Raymond

and Scheibling 1987, Balch and Scheibling unpubl. data) suggested that zygote production in the shallow subtidal zone must interact with other factors, such as larval transport and survival (Scheltema 1986, Hart and Scheibling 1988a), to determine sea urchin settlement patterns in this zone. I estimated that the number of zygotes produced by sea urchins in kelp beds was less than that required to initiate a population outbreak leading to destructive grazing.

My research achieved two main goals. Firstly, it enhanced our understanding of dietary effects on the reproductive ecology of *Strongylocentrotus droebachiensis*. The inclusion in my study of grazing fronts represented the first extensive documentation of reproduction of sea urchins in this transient subpopulation. Secondly, I developed a model that allowed me to determine the importance of reproductive processes to the population dynamics of *S. droebachiensis*. This model has identified deficiencies in our knowledge of the population ecology of *S. droebachiensis* and environmental conditions over its range that will guide future research on this species and its role in the subtidal ecosystem.

Literature cited

Aldana Aranda, D., Lucas, A., Brule, T., Salguero, E. and Rendon, F. (1989). Effects of temperature, algal food, feeding rate and density on the larval growth of the milk conch (*Strombus costatus*) in Mexico. Aquaculture 76: 361-371.

Andrew, N.L. (1986). The interaction between diet and density in influencing reproductive output in the echinoid *Evechinus chloroticus* (Val.). J. Exp. Mar. Biol. Ecol. 97: 63-79.

Andrew, N.L. and Choat, J.H. (1982). The influence of predation and conspecific adults on the abundance of juvenile *Evechinus chloroticus* (Echinoidea: Echinometridae). Oecologia 54: 80-87.

Anger, K. (1984). Influence of starvation on moult cycle and morphogenesis of *Hyas araneus* larvae (Decapoda, Majidae). Helgoländer wiss. Meeresunters. 38: 21-33.

Babcock, R.C. and Mundy, C.N. (1992). Reproductive biology, spawning and field fertilization rates of *Acanthaster planci*. Aust. J. Mar. Freshwater Res. 43: 525-534.

Babcock, R.C., Mundy, C.N. and Whitehead, D. (1994). Sperm diffusion models and in situ confirmation of long-distance fertilization in the free-spawning asteroid *Acanthaster planci*. Biol. Bull. 186: 17-28.

Balch, T. and Scheibling, R.E. (in press). Larval supply, settlement and recruitment in echinoderms. In: J. Lawrence and M. Jangoux (Eds.), Echinoderms studies 6. A.A. Balkema, Rotterdam.

Balch, T. and Scheibling, R.E. (1998). Settlement and recruitment of echinoderms in kelp beds and sea urchin-dominated barren grounds in Nova Scotia. In: R. Mooi and M. Telford (Eds.), Echinoderms: San Francisco. A.A. Balkema, Rotterdam, p. 553.

Balch, T., Scheibling, R.E., Harris, L.G., Chester, C.M. and Robinson, S.M.C. (1998). Variation in settlement of *Strongylocentrotus droebachiensis* in the northwest Atlantic: effects of spatial scale and sampling method. In: R. Mooi and M. Telford (Eds.), Echinoderms: San Francisco. A.A. Balkema, Rotterdam, pp. 555-560.

Basch, L.V. (1996). Effects of algal and larval densities on development and survival of asteroid larvae. Mar. Biol. 126: 693-701.

Basch, L.V. and Pearse, J.S. (1996). Consequences of larval feeding environment for settlement and metamorphosis of a temperate echinoderm. Oceanol. Acta 19: 273-285.

Bayne, B.L., Holland, D.L., Moore, M.N., Lowe, D.M. and Widdows, J. (1978). Further studies on the effects of stress in the adult on the eggs of *Mytilus edulis*. J. Mar. Biol. Ass. U.K. 58: 825-841.

Bennett, J. and Giese, A.C. (1955). The annual reproductive and nutritional cycles in two western sea urchins. Biol. Bull. 109: 226-237.

Bernard, F.R. (1977). Fishery and reproductive cycle of the red sea urchin, *Strongylocentrotus franciscanus*, in British Columbia. J. Fish. Res. Board Can. 34: 604-610.

Bernstein, B.B., Williams, B.E. and Mann, K.H. (1981). The role of behavioural responses to predators in modifying urchins' (*Strongylocentrotus droebachiensis*) destructive grazing and seasonal foraging patterns. Mar. Biol. 63: 39-49.

Bertram, D.F. and Strathmann, R.R. (1998). Effects of maternal and larval nutrition on growth and form of planktotrophic larvae. Ecology 79: 315-327.

Boidron-Metairon, I.F. (1988). Morphological plasticity in laboratory-reared echinoplutei of *Dendraster excentricus* (Eschscholtz) and *Lytechinus variegatus* (Lamarck) in response to food conditions. J. Exp. Mar. Biol. Ecol. 119: 31-41.

Boidron-Metairon, I.F. (1995). Larval nutrition. In: L.R. McEdward (Ed.), Ecology of marine invertebrate larvae. CRC Press, Inc., Boca Raton, pp. 223-248.

Branham, J.M., Reed, S.A. and Bailey, J.H. (1971). Coral-eating sea stars *Acanthaster planci* in Hawaii. Science 172: 1155-1157.

Brazeau, D.A. and Lasker, H.R. (1992). Reproductive success in the Caribbean octocoral *Briareum asbestinum*. Mar. Biol. 114: 157-163.

Breen, P.A. and Mann, K.H. (1976a). Changing lobster abundance and the destruction of kelp beds by sea urchins. Mar. Biol. 34: 137-142.

Breen, P.A. and Mann, K.H. (1976b). Destructive grazing of kelp by sea urchins in eastern Canada. J. Fish. Res. Board Can. 33: 1278-1283.

Briscoe, C.S. and Sebens, K.P. (1988). Omnivory in *Strongylocentrotus droebachiensis* (Müller) (Echinodermata: Echinoidea): predation on subtidal mussels. J. Exp. Mar. Biol. Ecol. 115: 1-24.

Buchanan, J.B. (1966). The biology of *Echinocardium cordatum* (Echinodermata: Spatangoidea) from different habitats. J. Mar. Biol. Ass. U.K. 46: 97-114.

Byrne, M. (1990). Annual reproductive cycles of the commercial sea urchin *Paracentrotus lividus* from an exposed intertidal and a sheltered subtidal habitat on the west coast of Ireland. Mar. Biol. 104: 275-289.

Carpenter, R.C. (1981). Grazing by *Diadema antillarum* (Philippi) and its effects on the benthic algal community. J. Mar. Res. 39: 749-765.

Chapman, A.R.O. (1981). Stability of sea urchin dominated barren grounds following destructive grazing of kelp in St. Margaret's Bay, eastern Canada. Mar. Biol. 62: 307-311.

Chapman, A.R.O. and Johnson, C.R. (1990). Disturbance and organization of macroalgal assemblages in the Northwest Atlantic. Hydrobiologia 192: 77-121.

Chatlynne, L.G. (1969). A histochemical study of oogenesis in the sea urchin, *Strongylocentrotus purpuratus*. Biol. Bull. 136: 167-184.

Chia, F.-S. and Bickell, L.R. (1983). Echinodermata. In: K.G. Adiyodi and R.G. Adiyodi (Eds.), Reproductive Biology of Invertebrates, Vol. II. John Wiley & Sons Ltd., New York, pp 545-620.

Clark, P.J. and Evans, F.C. (1954). Distance to nearest neighbor as a measure of spatial relationships in populations. Ecology 35: 445-453.

Cocanour, B. and Allen, K. (1967). The breeding cycles of a sand dollar and a sea urchin. Comp. Biochem. Physiol. 20: 327-331.

Coma, R. and Lasker, H.R. (1997). Effects of spatial distribution and reproductive biology on *in situ* fertilization rates of a broadcast-spawning invertebrate. Biol. Bull. 193: 20-29.

Crapp, G.B. and Willis, M.E. (1975). Age determination in the sea urchin *Paracentrotus lividus* (Lamarck), with notes on the reproductive cycle. J. Exp. Mar. Biol. Ecol. 20: 157-178.

Creaser, T. and Hunter, M. (1997). Commercial port sampling for sea urchins. Maine Department of Marine Resources, Annual Report of the Sea Urchin Program, pp. 1-10.

Csanady, G.S. (1973). Turbulent diffusion in the environment. D. Reidel Pub. Co., Boston.

Dayton, P.K. (1985). The structure and regulation of some South American kelp communities. Ecol. Monogr. 55: 447-468.

de Jong-Westman, M., March, B.E. and Carefoot, T.H. (1995a). The effect of different nutrient formulations in artificial diets on gonad growth in the sea urchin *Strongylocentrotus droebachiensis*. Can. J. Zool. 73: 1495-1502.

de Jong-Westman, M., Qian, P.-Y., March, B.E. and Carefoot, T.H. (1995b). Artificial diets in sea urchin culture: effects of dietary protein level and other additives on egg quality, larval morphometrics, and larval survival in the green sea urchin, *Strongylocentrotus droebachiensis*. Can. J. Zool. 73: 2080-2090.

De Ridder, C. and Lawrence, J.M. (1982). Food and feeding mechanisms: Echinoidea. In: M. Jangoux and J. M. Lawrence (Eds.), Echinoderm nutrition. A.A. Balkema, Rotterdam, pp. 57-115.

Denny, M.W. (1988). Biology and mechanics of the wave-swept environment. Princeton University Press, Princeton.

Denny, M.W. and Shibata, M.F. (1989). Consequences of surf-zone turbulence for settlement and external fertilization. Am. Nat. 134: 859-889.

Dix, T.G. (1970). Biology of *Evechinus chloroticus* (Echinoidea: Echinometridae) from different localities. 3. Reproduction. N.Z. J. Mar. Freshwater. Res. 4: 385-405.

Dix, T.G. (1972). Biology of *Evechinus chloroticus* (Echinoidea: Echinometridae) from different localities. 4. Age, growth, and size. N.Z. J. Mar. Freshwater Res. 6: 48-68.

Ebert, T.A. (1968). Growth rates of the sea urchin *Strongylocentrotus purpuratus* related to food availability and spine abrasion. Ecology 49: 1075-1091.

Eckman, J.E. (1983). Hydrodynamic processes affecting benthic recruitment. Limnol. Oceanogr. 28: 241-257.

Eckman, J.E., Duggins, D.O. and Sewell, A.T. (1989). Ecology of understory kelp environments. I. Effects of kelp on flow and particle transport near the bottom. J. Exp. Mar. Biol. Ecol. 129: 173-187.

Edelstein, T., Craigie, J.S. and McLachlan, J. (1969). Preliminary survey of the sublittoral flora of Halifax County. J. Fish. Res. Board Can. 26: 2703-2713.

Emson, R.H. and Moore, P.G. (1998). Diet and gonad size in three populations of *Echinus esculentus*. In: R. Mooi and M. Telford (Eds.), Echinoderms: San Francisco. A.A. Balkema, Rotterdam, pp. 641-644.

Falk-Petersen, I.-B. and Lønning, S. (1983). Reproductive cycles of two closely related sea urchin species, *Strongylocentrotus droebachiensis* (O.F. Müller) and *S. pallidus* (G.O. Sars). Sarsia 68: 157-164.

Fenaux, L., Strathmann, M.F. and Strathmann, R.R. (1994). Five tests of food-limited growth of larvae in coastal waters by comparisons of rates of development and form of echinoplutei. Limnol. Oceanogr. 39: 84-98.

Fernandez, C. and Caltagirone, A. (1994). Growth rate of adult sea urchins, *Paracentrotus lividus* in a lagoon environment: The effect of different diet types. In: B. David, A. Guille, J.-P. Féral and M. Roux (Eds.), Echinoderms through Time. A.A. Balkema, Rotterdam, pp. 655-660.

Fernandez, C. and Boudouresque, C.-F. (1997). Phenotypic plasticity of *Paracentrotus lividus* (Echinodermata: Echinoidea) in a lagoonal environment. Mar. Ecol. Prog. Ser. 152: 145-154.

Foreman, R.E. (1977). Benthic community modification and recovery following intensive grazing by *Strongylocentrotus droebachiensis*. Helgoländer wiss. Meeresunters. 30: 468-484.

Fuji, A. (1960a). Studies on the biology of the sea urchin. I. Superficial and histological gonadal changes in gametogenic process of two sea urchins, *Strongylocentrotus nudus* and *S. intermedius*. Bull. Fac. Fish., Hokkaido Univ. 11: 1-14.

Fuji, A. (1960b). Studies on the biology of the sea urchin. III. Reproductive cycles of two sea urchins, *Strongylocentrotus nudus* and *S. intermedius*, in Southern Hokkaido. Bull. Fac. Fish., Hokkaido Univ 11: 49-57.

Fuji, A. (1962). Studies on the biology of the sea urchin. V. Food consumption of *Strongylocentrotus intermedius*. Jap. J. Ecol. 12: 181-186.

Gaines, S.D. and Lafferty, K.D. (1995). Modeling the dynamics of marine species: the importance of incorporating larval dispersal. In: L. R. McEdward (Ed.), Ecology of marine invertebrate larvae. CRC Press, Inc., Boca Raton, pp. 389-412.

George, S.B. (1990). Population and seasonal differences in egg quality of *Arbacia lixula* (Echinodermata: Echinoidea). Invert. Reprod. Develop. 17: 111-121.

George, S.B. (1996). Echinoderm egg and larval quality as a function of adult nutritional state. Oceanol. Acta 19: 297-308.

George, S.B., Cellario, C. and Fenaux, L. (1990). Population differences in egg quality of *Arbacia lixula* (Echinodermata: Echinoidea): proximate composition of eggs and larval development. J. Exp. Mar. Biol. Ecol. 141: 107-118.

George, S.B., Lawrence, J.M. and Fenaux, L. (1991). The effect of food ration on the quality of eggs of *Luidia clathrata* (Say) (Echinodermata: Asteroidea). Invert. Reprod. Develop. 20: 237-242.

George, S.B., Young, C.M. and Fenaux, L. (1997). Proximate composition of eggs and larvae of the sand dollar *Encope michelini* (Agassiz): the advantage of higher investment in planktotrophic eggs. Invert. Reprod. Develop. 32: 11-19.

Gerard, V.A. and Mann, K.H. (1979). Growth and production of *Laminaria longicruris* (Phaeophyta) populations exposed to different intensities of water movement. J. Phycol. 15: 33-41.

Gonor, J.J. (1972). Gonad growth in the sea urchin, *Strongylocentrotus purpuratus* (Stimpson) (Echinodermata: Echinoidea) and the assumptions of gonad index methods. J. Exp. Mar. Biol. Ecol. 10: 89-103.

Gonor, J.J. (1973a). Reproductive cycles in Oregon populations of the echinoid, *Strongylocentrotus purpuratus* (Stimpson). I. Annual gonad growth and ovarian gametogenic cycles. J. Exp. Mar. Biol. Ecol. 12: 45-64.

Gonor, J.J. (1973b). Reproductive cycles in Oregon populations of the echinoid, *Strongylocentrotus purpuratus* (Stimpson). II. Seasonal changes in oocyte growth and in abundance of gametogenic stages in the ovary. J. Exp. Mar. Biol. Ecol. 12: 65-78.

Gonor, J.J. (1973c). Sex ratio and hermaphroditism in Oregon intertidal populations of the echinoid *Strongylocentrotus purpuratus*. Mar. Biol. 19: 278-280.

Gonzalez, L.P., Castilla, J.C. and Guisado, C. (1987). Effect of larval diet and rearing temperature on metamorphosis and juvenile survival of the edible sea urchin *Loxechinus albus* (Molina, 1782) (Echinoidea, Echinidae). J. Shellfish Res. 6: 109-115.

Grosberg, R.K. (1991). Sperm-mediated gene flow and the genetic structure of a population of the colonial ascidian *Botryllus schlosseri*. Evolution 45: 130-142.

Grosberg, R.K. and Levitan, D.R. (1992). For adults only? Supply-side ecology and the history of larval biology. Trends Ecol. Evol. 7: 130-133.

Guillou, M. and Michel, C. (1993). Reproduction and growth of *Sphaerechinus granularis* (Echinodermata: Echinoidea) in Southern Brittany. J. mar. biol. Assoc. UK 73: 79-192.

Guisande, C. and Harris, R. (1995). Effect of total organic content of eggs on hatching success and naupliar survival in the copepod *Calanus helgolandicus*. Limnol. Oceanogr. 40: 476-482.

Hagen, N.T. (1983). Destructive grazing of kelp beds by sea urchins in Vestfjorden, Northern Norway. Sarsia 68: 177-190.

Harrold, C. and Reed, D.C. (1985). Food availability, sea urchin grazing, and kelp forest community structure. Ecology 66: 1160-1169.

Harrold, C. and Pearse, J.S. (1987). The ecological role of echinoderms in kelp forests. In: M. Jangoux and J. M. Lawrence (Eds.), Echinoderm Studies 2. A.A. Balkema, Rotterdam, pp. 137-233.

Hart, M.W. and Scheibling, R.E. (1988a). Heat waves, baby booms, and the destruction of kelp beds by sea urchins. Mar. Biol. 99: 167-176.

Hart, M.W. and Scheibling, R.E. (1988b). Comparing shapes of echinoplutei using principal components analysis, with an application to larvae of *Strongylocentrotus droebachiensis*. In: R. D. Burke, P. V. Mladenov, P. Lambert and R. L. Parsley (Eds.), Echinoderm Biology, A.A. Balkema, Rotterdam, pp. 277-284.

Hart, M.W. and Strathmann, R.R. (1994). Functional consequences of phenotypic plasticity in echinoid larvae. Biol. Bull. 186: 291-299.

Hatcher, B.G. and Hatcher, A.I. (1997). Research directions and management options for sea urchin culture in Nova Scotia. Bull. Aquacul. Assoc. Canada 97: 62-65.

Himmelman, J.H. (1975). Phytoplankton as a stimulus for spawning in three marine invertebrates. J. Exp. Mar. Biol. Ecol. 20: 199-214.

Himmelman, J.H. (1978). Reproductive cycle of the green sea urchin, *Strongylocentrotus droebachiensis*. Can. J. Zool. 56: 1828-1836.

Himmelman, J.H. (1980). The role of the green sea urchin, *Strongylocentrotus droebachiensis*, in the rocky subtidal region of Newfoundland. In: J. D. Pringle, G. J. Sharp and J. F. Caddy (Eds.), Proceedings of the workshop on the relationship between sea urchin gazing and commercial plant/animal harvesting. Can. Tech. Rep. Fish. Aquat. Sci. 954: 92-119.

Himmelman, J.H. (1984). Urchin feeding and macroalgal distribution in Newfoundland, Eastern Canada. Nat. Can. 111: 337-348.

Himmelman, J.H. (1986). Population biology of green sea urchins on rocky bottoms. Mar. Ecol. Prog. Ser. 33: 295-306.

Himmelman, J.H. and Steele, D.H. (1971). Foods and predators of the green sea urchin *Strongylocentrotus droebachiensis* in Newfoundland waters. Mar. Biol. 9: 315-322.

Himmelman, J.H., Cardinal, A. and Bourget, E. (1983a). Community development following removal of urchins, *Strongylocentrotus droebachiensis*, from the rocky subtidal zone of the St. Lawrence Estuary, Eastern Canada. Oecologia 59: 27-39.

Himmelman, J.H., Lavergne, Y., Axelsen, F., Cardinal, A. and Bourget, E. (1983b). Sea urchins in the St. Lawrence estuary: their abundance, size-structure, and suitability for commercial exploitation. Can. J. Fish. Aquat. Sci. 40: 474-486.

Himmelman, J.H. and Nédélec, H. (1990). Urchin foraging and algal survival strategies in intensely grazed communities in Eastern Canada. Can. J. Fish. Aquat. Sci. 47: 1011-1026.

Hjörleifsson, E., Kaasa, Ö. and Gunnarsson, K. (1995). Grazing of kelp by green sea urchin in Eyjafjördur, North Iceland. In: H. R. Skjoldal, C. Hopkins, K. E. Erikstad and H. P. Leinaas (Eds.), Ecology of fjords and coastal waters. Elsevier Science B.V., Amsterdam, pp. 593-597.

Howell, D.C. (1987). Statistical methods for psychology, 2nd ed. Duxbury Press, Boston.

Jackson, G.A. (1986). Interaction of physical and biological processes in the settlement of planktonic larvae. Bull. Mar. Sci. 39: 202-212.

Jensen, M. (1969). Age determination of echinoids. Sarsia 37: 41-44.

Johnson, C.R. and Mann, K.H. (1982). Adaptations of *Strongylocentrotus droebachiensis* for survival on barren grounds in Nova Scotia. In: J. M. Lawrence (Ed.), Echinoderms: Proceedings of the International Conference, Tampa Bay. A.A. Balkema, Rotterdam, pp. 277-283.

Johnson, C.R. and Mann, K.H. (1986). The crustose coralline alga, *Phymatolithon* Foslie, inhibits the overgrowth of seaweeds without relying on herbivores. J. Exp. Mar. Biol. Ecol. 96: 127-146.

Johnson, C.R. and Mann, K.H. (1988). Diversity, patterns of adaptation, and stability of Nova Scotian kelp beds. Ecol. Monogr. 58: 129-154.

Jones, G.M. (1985). *Paramoeba invadens* n.sp. (Amoebida, Paramoebidae), a pathogenic amoeba from the sea urchin, *Strongylocentrotus droebachiensis*, in eastern Canada. J. Protozool. 32: 564-569.

Jones, G.M. and Scheibling, R.E. (1985). *Paramoeba* sp. (Amoebida, Paramoebidae) as the possible causative agent of sea urchin mass mortality in Nova Scotia. J. Parasit. 71: 559-565.

Kawamata, S. (1997). Modelling the feeding rate of the sea urchin *Strongylocentrotus nudus* (A. Agassiz). J. Exp. Mar. Biol. Ecol. 210: 107-127.

Keats, D.M., Steele, D.H. and South, G.R. (1983). Food relations and short term aquaculture potential of the green sea urchin (*Strongylocentrotus droebachiensis*) in Newfoundland. Meml. Univ. Newfoundland Mar. Sci. Res. Lab. Tech. Rep. 24: 1-24.

Keats, D.W., Steele, D.H. and South, G.R. (1984). Depth-dependent reproductive output of the green sea urchin, *Strongylocentrotus droebachiensis* (O.F. Müller), in relation to the nature and availability of food. J. Exp. Mar. Biol. Ecol. 80: 77-91.

Keats, D.M., South, G.R. and Steele, D.H. (1985). Ecology of juvenile green sea urchins (*Strongylocentrotus droebachiensis*) at an urchin dominated sublittoral site in eastern Newfoundland. In: B. F. Keegan and B. D. S. O'Connor (Eds.), Echinodermata. A.A. Balkema, Rotterdam, pp. 295-302.

Keats, D.W., Hooper, R.G., Steele, D.H. and South, G.R. (1987). Field observations of summer and autumn spawning by *Strongylocentrotus droebachiensis*, green sea urchins, in eastern Newfoundland. Can. Field Nat. 101: 463-465.

King, C.K., Hoegh-Guldberg, O. and Byrne, M. (1994). Reproduction cycle of *Centrostephanus rodgersii* (Echinoidea), with recommendations for the establishment of a sea urchin fishery in New South Wales. Mar. Biol. 120: 95-106.

Lamare, M.D. and Stewart, B.G. (1998). Mass spawning by the sea urchin *Evechinus chloroticus* (Echinodermata: Echinoidea) in a New Zealand fjord. Mar. Biol. 132: 135-140.

Lang, C. and Mann, K.H. (1976). Changes in sea urchin populations after the destruction of kelp beds. Mar. Biol. 36: 321-326.

Larson, B.R., Vadas, R.L. and Keser, M. (1980). Feeding and nutritional ecology of the sea urchin *Strongylocentrotus drobachiensis* in Maine, USA. Mar. Biol. 59: 49-62.

Lasker, R. and Giese, A.C. (1954). Nutrition of the sea urchin, *Strongylocentrotus purpuratus*. Biol. Bull. 106: 328-340.

Lawrence, J.M. (1975). On the relationships between marine plants and sea urchins. Oceanogr. Mar. Biol. Ann. Rev. 13: 213-286.

Lawrence, J.M. (1987). A functional biology of echinoderms. The Johns Hopkins University Press, Baltimore.

Lawrence, J.M., Lawrence, A.L. and Holland, N.D. (1965). Annual cycle in the size of the gut of the purple sea urchin, *Strongylocentrotus purpuratus* (Stimpson). Nature 205: 1238-1239.

Lawrence, J., Fenaux, L., Corre, M.C. and Lawrence, A. (1992). The effects of quantity and quality of prepared diets on production in *Paracentrotus lividus* (Echinodermata: Echinoidea). In: L. Scalera-Liaci and C. Canicatti (Eds.), Echinoderm Research 1991. A.A. Balkema, Rotterdam, pp. 107-110.

Lawrence, J., Lumingas, L. and Guillou, M. (1994). Plasticité de l'oursin *Sphaerechinus granularis* (Lamarck), face aux variations de l'environnement. In: B. David, A. Guille, J.-P. Féral and M. Roux (Eds.), Echinoderms through time. A.A. Balkema, Rotterdam, pp. 757-763.

Lee, Y.Z. and Haard, N.F. (1982). Evaluation of the green sea urchin gonads as a food source. Can. Inst. Food Sci. Technol. J. 15: 233-235.

Leinaas, H.P. and Christie, H. (1996). Effects of removing sea urchins (*Strongylocentrotus droebachiensis*): stability of the barren state and succession of kelp forest recovery in the east Atlantic. Oecologia 105: 524-536.

Lemire, M. and Himmelman, J.H. (1996). Relation of food preference to fitness for the green sea urchin, *Strongylocentrotus droebachiensis*. Mar. Biol. 127: 73-78.

Levin, V.S. and Naidenko, V.P. (1987). Artificial diets for the laboratory-maintained sea urchin *Strongylocentrotus intermedius*. Biol. Morya 6: 50-56.

Levitan, D.R. (1988a). Density-dependent size regulation and negative growth in the sea urchin *Diadema antillarum* Philippi. Oecologia 76: 627-629.

Levitan, D.R. (1988b). Asynchronous spawning and aggregative behaviour in the sea urchin *Diadema antillarum* (Philippi). In: R. D. Burke, P. V. Mladenov, P. Lambert and R. L. Parsley (Eds.), Echinoderm Biology. A.A. Balkema, Rotterdam, pp. 181-186.

Levitan, D.R. (1991). Influence of body size and population density on fertilization success and reproductive output in a free-spawning invertebrate. Biol. Bull. 181: 261-268.

Levitan, D.R. (1993). The importance of sperm limitation to the evolution of egg size in marine invertebrates. Am. Nat. 141: 517-536.

Levitan, D.R. (1995). The ecology of fertilization in free-spawning invertebrates. In: L. R. McEdward (Ed.), Ecology of Marine Invertebrate Larvae. CRC Press, Inc., Boca Raton, pp. 123-156.

Levitan, D.R., Sewell, M.A. and Chia, F.-S. (1992). How distribution and abundance influence fertilization success in the sea urchin *Strongylocentrotus franciscanus*. Ecology 73: 248-254.

Levitan, D.R. and Young, C.M. (1995). Reproductive success in large populations: empirical measures and theoretical predictions of fertilization in the sea biscuit *Clypeaster rosaceus*. J. Exp. Mar. Biol. Ecol. 190: 221-241.

Logan, A., Page, F.H. and Thomas, M.L.H. (1984). Depth zonation of epibenthos on sublittoral hard substrates off Deer Island, Bay of Fundy, Canada. Est. Coast. Shelf Sci. 18: 571-592.

Lucas, J.S. (1982). Quantitative studies of feeding and nutrition during larval development of the coral reef asteroid *Acanthaster planci* (L.). J. Exp. Mar. Biol. Ecol. 65: 173-193.

Lucas, C.H. and Lawes, S. (1998). Sexual reproduction of the scyphomedusa *Aurelia aurita* in relation to temperature and variable food supply. Mar. Biol. 131: 629-638.

Mann, K.H. (1972). Ecological energetics of the seaweed zone in a marine bay on the Atlantic coast of Canada. I. Zonation and biomass of seaweeds. Mar. Biol. 12: 1-10.

Mann, K.H. (1977). Destruction of kelp-beds by sea urchins: a cyclical phenomenon or irreversible degradation? Helgoländer wiss. Meeresunters. 30: 455-467.

Mann, K.H. (1985). Invertebrate behaviour and the structure of marine benthic communities. In: R. M. Sibley and R. H. Smith (Eds.), Behavioural ecology. Blackwell Scientific Publications, Oxford, pp. 227-246.

Mann, K.H., Wright, J.L.C., Welsford, B.E. and Hatfield, E. (1984). Responses of the sea urchin *Strongylocentrotus droebachiensis* (O.F. Müller) to water-borne

stimuli from potential predators and potential food algae. J. Exp. Mar. Biol. Ecol. 79: 233-244.

Martin, P.D., Truchon, S.P. and Harris, L.G. (1988). *Strongylocentrotus droebachiensis* populations and community dynamics at two depth-related zones over an 11-year period. In: R. D. Burke, P. V. Mladenov, P. Lambert and R. L. Parsley (Eds.), Echinoderm Biology. A.A. Balkema, Rotterdam, pp. 475-482.

Martinez, P.C. and Richmond, R.H. (1998). Effect of diet on growth and larval development of the sea cucumber *Holothuria nobilis* in Guam. In: R. Mooi and M. Telford (Eds.), Echinoderms: San Francisco. A.A. Balkema, Rotterdam, p. 480.

Mattison, J.E., Trent, J.D., Shanks, A.L., Akin, T.B. and Pearse, J.S. (1977). Movement and feeding activity of red sea urchins (*Strongylocentrotus franciscanus*) adjacent to a kelp forest. Mar. Biol. 39: 25-30.

McLaughlin, M.W., Cheney, D.P., Goldhor, S. and Giurca, R. (1996). Building a sustainable sea urchin fishery in Massachusetts. 24th Annual Benthic Ecology Meeting, The University of South Carolina, Columbia, p. 58.

Medeiros-Bergen, D.E., Olson, R.R., Conroy, J.A. and Kocher, T.D. (1995). Distribution of holothurian larvae determined with species-specific probes. Limnol. Oceanogr. 40: 1225-1235.

Miller, R.J. (1985a). Succession in sea urchin and seaweed abundance in Nova Scotia, Canada. Mar. Biol. 84: 275-286.

Miller, R.J. (1985b). Seaweeds, sea urchins, and lobster: a reappraisal. Can. J. Fish. Aquat. Sci. 42: 2061-2072.

Miller, R.J. and Mann, K.H. (1973). Ecological energetics of the seaweed zone in a marine bay on the Atlantic coast of Canada. III. Energy transformations by sea urchins. Mar. Biol. 18: 99-114.

Miller, R.J. and Colodey, A.G. (1983). Widespread mass mortalities of the green sea urchin in Nova Scotia, Canada. Mar. Biol. 73: 263-267.

Minchinton, T.E. and Scheibling, R.E. (1991). The influence of larval supply and settlement on the population structure of barnacles. Ecology 72: 1867-1879.

Minor, M.A. and Scheibling, R.E. (1997). Effects of food ration and feeding regime on growth and reproduction of the sea urchin *Strongylocentrotus droebachiensis*. Mar. Biol. 129: 159-167.

Moore, D.S. and Miller, R.J. (1983). Recovery of macroalgae following widespread sea urchin mortality with a description of the nearshore hard-bottom habitat on the Atlantic coast of Nova Scotia. Can. Tech. Rep. Fish. Aquat. Sci. 1230: vii + 94 pp.

Moore, D.S., Miller, R.J. and Meade, L.D. (1986). Survey of shallow benthic habitat: Eastern shore and Cape Breton, Nova Scotia. Can. Tech. Rep. Fish. Aquat. Sci. 1546: v + 49 pp.

Moore, H.B. (1934). A comparison of the biology of *Echinus esculentus* in different habitats. Part I. J. mar. biol. Ass. U.K. 19: 869-885.

Morgan, S.G. (1995). Life and death in the plankton: larval mortality and adaptation. In: L. R. McEdward (Ed.), Ecology of marine invertebrate larvae. CRC Press, Inc., Boca Raton, pp. 279-321.

Munk, J.E. (1992). Reproduction and growth of green sea urchins *Strongylocentrotus droebachiensis* (Müller) near Kodiak, Alaska. J. Shellfish Res. 11: 245-254.

Nestler, E.C. and Harris, L.G. (1994). The importance of omnivory in *Strongylocentrotus droebachiensis* (Müller) in the Gulf of Maine. In: B. David, A. Guille, J.-P. Féral and M. Roux (Eds.), Echinoderms through time. A.A. Balkema, Rotterdam, pp. 813-818.

Novaczek, I. and McLachlan, J. (1986). Recolonization by algae of the sublittoral habitat of Halifax county, Nova Scotia, following the demise of sea urchins. Bot. Mar. 29: 69-73.

Oliver, J. and Babcock, R. (1992). Aspects of the fertilization ecology of broadcast spawning corals: sperm dilution effects and *in situ* measurements of fertilization. Biol. Bull. 183: 409-417.

Paine, R.T. and Vadas, R.L. (1969). The effects of grazing by sea urchins, *Strongylocentrotus* spp, on benthic algal populations. Limnol. Oceanogr. 14: 710-719.

Palumbi, S.R. (1995). Using genetics as an indirect estimator of larval dispersal. In: L. R. McEdward (Ed.), Ecology of marine invertebrate larvae. CRC Press, Inc., Boca Raton, pp. 369-387.

Paulay, G., Boring, L. and Strathmann, R.R. (1985). Food limited growth and development of larvae: experiments with natural sea water. J. Exp. Mar. Biol. Ecol. 93: 1-10.

Pearce, C.M. and Scheibling, R.E. (1990). Induction of metamorphosis of larvae of the green sea urchin, *Strongylocentrotus droebachiensis*, by coralline red algae. Biol. Bull. 179: 304-311.

Pearce, C.M. and Scheibling, R.E. (1991). Effect of macroalgae, microbial films, and conspecifics on the induction of metamorphosis of the green sea urchin *Strongylocentrotus droebachiensis*. J. Exp. Mar. Biol. Ecol. 47: 147-162.

Pearse, J.S. (1980). Synchronization of gametogenesis in the sea urchins *Strongylocentrotus purpuratus* and *S. franciscanus*. In: W. H. J. Clark and T.

S. Adams (Eds.), Advances in invertebrate reproduction. Elsevier North Holland, Inc., New York, pp. 53-68.

Pearse, J.S. and Hines, A.H. (1987). Long-term population dynamics of sea urchins in a central California kelp forest: rare recruitment and rapid decline. Mar. Ecol. Prog. Ser. 39: 275-283.

Pearse, J.S., McClary, D.J., Sewell, M.A., Austin, W.C., Perez-Ruzafa, A. and Byrne, M. (1988). Simultaneous spawning of six species of echinoderms in Barkley Sound, BC. Invert Reprod Develop 14: 279-288.

Pennington, J.T. (1985). The ecology of fertilization of echinoid eggs: the consequences of sperm dilution, adult aggregation, and synchronous spawning. Biol. Bull. 169: 417-430.

Pennington, J.T., Rumrill, S.S. and Chia, F.-S. (1986). Stage-specific predation upon embryos and larvae of the Pacific sand dollar, *Dendraster excentricus*, by 11 species of common zooplanktonic predators. Bull. Mar. Sci. 39: 234-240.

Propp, M.V. (1977). Ecology of the sea urchin *Strongylocentrotus droebachiensis* of the Barents Sea: metabolism and regulation of abundance. Soviet J. mar. Biol 3: 27-37.

Qian, P.-Y. (1994). Effect of food quantity on growth and reproductive characteristics of *Capitella* sp. (Annelida: Polychaeta). Invert. Reprod. Develop. 26: 175-185.

Raymond, B.G. and Scheibling, R.E. (1987). Recruitment and growth of the sea urchin *Strongylocentrotus droebachiensis* following mass mortalities off Nova Scotia, Canada. J. Exp. Mar. Biol. Ecol. 108: 31-54.

Reyment, R.A., Blacksmith, R.E. and Campbell, N.A. (1984). Multivariate morphometrics, 2nd ed. Academic Press, London.

Robinson, S.M.C. and MacIntyre, A.D. (1997). Aging and growth of the green sea urchin. Bull. Aquacul. Assoc. Canada 97: 56-60.

Rogers-Bennett, L., Bennett, W.A., Fastenau, H.C. and Dewees, C.M. (1995). Spatial variation in red sea urchin reproduction and morphology: implications for harvest refugia. Ecol. Appl. 5: 1171-1180.

Rowley, R.J. (1989). Settlement and recruitment of sea urchins (*Strongylocentrotus* spp.) in a sea-urchin barren ground and a kelp bed: are populations regulated by settlement or post-settlement processes? Mar. Biol. 100: 485-494.

Rowley, R.J. (1990). Newly settled sea urchins in a kelp bed and urchin barren ground: a comparison of growth and mortality. Mar. Ecol. Prog. Ser. 62: 229-240.

Rumrill, S.S. and Chia, F.-S. (1984). Differential mortality during the embryonic and larval lives of northeast Pacific echinoids. In: B. F. Keegan and B. D. S. O'Connor (Eds.), Echinodermata. A.A. Balkema, Rotterdam, pp. 333-338.

Rumrill, S.S., Pennington, J.T. and Chia, F.-S. (1985). Differential susceptibility of marine invertebrate larvae: laboratory predation of sand dollar, *Dendraster excentricus* (Eschscholtz), embryos and larvae by zoeae of the red crab, *Cancer productus* Randall. J. Exp. Mar. Biol. Ecol. 90: 192-208.

Scheibling, R.E. (1981). The annual reproductive cycle of *Oreaster reticulatus* (L.) (Echinodermata: Asteroidea) and interpopulation differences in reproductive capacity. J. exp. mar Biol. Ecol. 54: 39-54.

Scheibling, R.E. (1984). Echinoids, epizootics and ecological stability in the rocky subtidal off Nova Scotia, Canada. Helgoländer wiss. Meeresunters. 37: 233-242.

Scheibling, R.E. (1986). Increased macroalgal abundance following mass mortalities of sea urchins (*Strongylocentrotus droebachiensis*) along the Atlantic coast of Nova Scotia. Oecologia 68: 186-198.

Scheibling, R.E. (1994). Interactions among lobsters, sea urchins, and kelp in Nova Scotia, Canada. In: B. David, A. Guille, J.-P. Féral and M. Roux (Eds.), Echinoderms through time. A.A. Balkema, Rotterdam, pp. 865-870.

Scheibling, R.E. (1996). The role of predation in regulating sea urchin populations in eastern Canada. Oceanol. Acta 19: 421-430.

Scheibling, R.E. and Stephenson, R.L. (1984). Mass mortality of *Strongylocentrotus droebachiensis* (Echinodermata: Echinoidea) off Nova Scotia, Canada. Mar. Biol. 78: 153-164.

Scheibling, R.E. and Raymond, B.G. (1990). Community dynamics on a subtidal cobble bed following mass mortalities of sea urchins. Mar. Ecol. Prog. Ser. 63: 127-145.

Scheibling, R.E., Hennigar, A.W. and Balch, T. (1994). The dynamics of destructive grazing of kelp beds by sea urchins in Nova Scotia. In: B. David, A. Guille, J.-P. Féral and M. Roux (Eds.), Echinoderms through time. A.A. Balkema, Rotterdam, pp. 871.

Scheibling, R.E. and Hennigar, A.W. (1997). Recurrent outbreaks of disease in sea urchins *Strongylocentrotus droebachiensis* in Nova Scotia: evidence for a link with large scale meteorologic and oceanographic events. Mar. Ecol. Prog. Ser. 152: 155-165.

Scheibling, R.E., Hennigar, A.W. and Balch, T. (submitted). Destructive grazing of kelp (*Laminaria longicruris*) by sea urchins (*Strongylocentrotus droebachiensis*) and the dynamics of the rocky subtidal community in Nova Scotia. Can. J. Fish. Aquat. Sci.

Scheltema, R.S. (1986). On dispersal and planktonic larvae of benthic invertebrates: an eclectic overview and summary of problems. Bull. Mar. Sci. 39: 290-322.

Shanks, A.L. (1995). Mechanisms of cross-shelf dispersal of larval invertebrates and fish. In: L. R. McEdward (Ed.), Ecology of marine invertebrate larvae. CRC Press, Inc., Boca Raton, pp. 323-367.

Sebens, K.P. (1985). The ecology of the rocky subtidal zone. Am. Sci. 73: 548-557.

Sivertsen, K. and Hopkins, C.C.E. (1995). Demography of the echinoid *Strongylocentrotus droebachiensis* related to biotope in northern Norway. In: H. R. Skjoldal, C. Hopkins, K. E. Erikstad and H. P. Leinaas (Eds.), Ecology of fjords and coastal waters. Elsevier Science B.V., Amsterdam, pp. 549-571.

Sokal, R.R. and Rohlf, F.J. (1995). Biometry, 3rd edn. W.H. Freeman and Company, New York.

Starr, M., Himmelman, J.H. and Therriault, J.-C. (1990). Direct coupling of marine invertebrate spawning with phytoplankton blooms. Science 247: 1071-1074.

Starr, M., Himmelman, J.H. and Therriault, J.-C. (1992). Isolation and properties of a substance from the diatom *Phaeodactylum tricornutum* which induces spawning in the sea urchin *Strongylocentrotus droebachiensis*. Mar. Ecol. Prog. Ser. 79: 275-287.

Starr, M., Himmelman, J.H. and Therriault, J.-C. (1993). Environmental control of green sea urchin, *Strongylocentrotus droebachiensis*, spawning in the St. Lawrence estuary. Can. J. Fish. Aquat. Sci. 50: 894-901.

Strathmann, R. (1978). Length of pelagic period in echinoderms with feeding larvae from the northwest Pacific. J. Exp. Mar. Biol. Ecol. 34: 23-27.

Strathmann, R.R., Fenaux, L. and Strathmann, M.F. (1992). Heterochronic developmental plasticity in larval sea urchins and its implications for evolution of nonfeeding larvae. Evolution 46: 972-986.

Strathmann, R.R., Fenaux, L., Sewell, A.T. and Strathmann, M.F. (1993). Abundance of food affects relative size of larval and postlarval structures of a molluscan veliger. Biol. Bull. 185: 232-239.

Swan, E.F. (1961). Some observations on the growth rate of sea urchins in the genus *Strongylocentrotus*. Biol. Bull. 120: 420-427.

Thomas, F.I.M. (1994). Physical properties of gametes in three sea urchin species. J. Exp. Biol. 194: 263-284.

Thompson, H.R. (1956). Distribution of distance to nth neighbour in a population of randomly distributed individuals. Ecology 37: 391-394.

Thompson, R.J. (1979). Fecundity and reproductive effort in the blue mussel (*Mytilus edulis*), the green sea urchin (*Strongylocentrotus droebachiensis*), and the snow

crab (*Chionoecetes opilio*) from populations in Nova Scotia and Newfoundland. J. Fish. Res. Board Can. 36: 955-964.

Thompson, R.J. (1982). The relationship between food ration and reproductive effort in the green sea urchin, *Strongylocentrotus droebachiensis*. Oecologia 56: 50-57.

Thompson, R.J. (1984). Partitioning of energy between growth and reproduction in three populations of the sea urchin *Strongylocentrotus droebachiensis*. In: W. Engels (Ed.), Advances in invertebrate reproduction 3. Elsevier Science Publ. B.V., pp. 425-431.

Thorson, G. (1950). Reproductive and larval ecology of marine bottom invertebrates. Biol. Rev. 25: 1-45.

Vadas, R.L. (1977). Preferential feeding: an optimization strategy in sea urchins. Ecol. Monogr. 47: 337-371.

Vance, R.R. and Schmitt, R.J. (1979). The effect of the predator-avoidance behavior of the sea urchin, *Centrostephanus coronatus*, on the breadth of its diet. Oecologia 44: 21-25.

Vogel, H., Czihak, G., Chang, P. and Wolf, W. (1982). Fertilization kinetics of sea urchin eggs. Math Biosci 58: 189-216.

Wahle, R.A. and Peckham, H. (in press). Density-related reproductive trade-offs in the green sea urchin *Strongylocentrotus droebachiensis*. Mar. Biol.

Wharton, W.G. (1980a). The relationship between destructive grazing by the sea urchin *Strongylocentrotus droebachiensis* and the abundance of American lobster *Homarus americanus* on the Atlantic coast of Nova Scotia. M.Sc. thesis, Dalhousie University, Halifax, Nova Scotia, Canada.

Wharton, W.G. (1980b). The distribution of sea urchin-dominated barren grounds along the south shore of Nova Scotia. In: J. D. Pringle, G. J. Sharp and J. F. Caddy (Eds.), Proceedings of the workshop on the relationship between sea urchin grazing and commercial plant/animal harvesting. Can. Tech. Rep. Fish. Aquat. Sci. 954: 33-47.

Wharton, W.G. and Mann, K.H. (1981). Relationship between destructive grazing by the sea urchin, *Strongylocentrotus droebachiensis*, and the abundance of American lobster, *Homarus americanus*, on the Atlantic coast of Nova Scotia. Can. J. Fish. Aquat. Sci. 38: 1339-1349.

Witman, J.D. (1985). Refuges, biological disturbance, and rocky subtidal community structure in New England. Ecol. Monogr. 55: 421-445.

Witman, J.D., Hulbert, A.H. and Harris, L.G. (1982). Morphology of a sea urchin front (*Strongylocentrotus droebachiensis*) at the Isles of Shoals, N.H. In: J. M. Lawrence (Ed.), Echinoderms: Proceedings of the International Conference, Tampa Bay. A.A. Balkema, Rotterdam, p. 529.

Young, C.M., Tyler, P.A., Cameron, J.L. and Rumrill, S.G. (1992). Seasonal breeding aggregations in low-density populations of the bathyal echinoid *Stylocidaris lineata*. Mar Biol 113: 603-612.

Yund, P.O. (1995). Gene flow via the dispersal of fertilizing sperm in a colonial ascidian (*Botryllus schlosseri*): the effect of male density. Mar. Biol. 122: 649-654.

Yund, P.O. (1998). The effect of sperm competition on male gain curves in a colonial marine invertebrate. Ecology 79: 328-339.

Yund, P.O. and McCartney, M.A. (1994). Male reproductive success in sessile invertebrates: competition for fertilizations. Ecology 75: 2151-2167.

Zar, J.H. (1984). Biostatistical analysis, 2nd ed. Prentice-Hall Inc., Englewood Cliffs.